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REVIEW ARTICLE

The Potential of Alkaloids in Drug Discovery

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Alkaloids are an important group of diversely distributed, chemically, biologically and commercially significant natural products. This article suggests why now, with the presently available technology, and the remaining biome available and reasonably accessible, is an opportune moment to consciously focus on the discovery of further alkaloids with pharmacophoric utility.

Keywords: alkaloids; drug discovery; taxonomic distribution; biological evaluation; future developments.

Introduction

'We need to talk'. When someone says that to you, it usually means that they have something quite serious on their mind. They are wanting to convey to you the profound seriousness and urgency of the matter at hand, and they are seeking to achieve your undivided attention, consideration, and input. So, with time passing, 'we need to talk'.

It is estimated that we are presently at a global human population of approximately 6.1 billion. Within 50 years, barring calamitous famine, disease, climactic change or extreme pestilence, our planet Earth will need to provide water, food, shelter, energy, consumable goods, and health care for a population in excess of 9 billion (Bureau of Census, 2000). At what point in time will we begin to seriously discuss how all of those needs will be achieved in a responsible manner within Gaia, our interdependent planet? What are the steps that we need to take now for the well-being of our descendants (Cordell, 1992), and what are the corresponding resource implications? As has been discussed elsewhere with respect to the provision of pharmaceutical agents from the biome (Wilson, 1988; Akerele *et al.*, 1991; Balick *et al.*, 1996; Cordell, 2000), we are cognizant of a number of global changes occurring which are operating against the full consideration of the awesome tasks ahead. In order to place the relationship of alkaloids to our future needs in an appropriate perspective, let us briefly discuss a selection of those factors.

Over the past 25 years, several studies have demonstrated, in a clear and categorical manner, the overall importance of natural products in the pharmaceutical market place. The earliest studies were those of Farnsworth and Morris (1976) for community pharmacy-based prescription products which showed about 50% of these were natural product-derived entities, half of them from plants. O'Neill and Lewis (1993) have indicated that half of the leading pharmaceuticals in 1991 based on sales were either natural product derived or contained a pharmacophore which was natural product based. A recent study (Grifo *et al.*, 1997) analysed the top 150 proprietary drugs from the National Prescription Audit (USA) for the period January to September 1993 and found that, based on prescription numbers, 57% of the top 150 brand name products prescribed contained at least one major active compound now or once derived or patterned after compounds derived from biological diversity. Seventeen percent were unmodified natural products. A study by De Smet (De Smet, 1997) of the 25 drugs with the largest sales in Dutch pharmacies in 1996 showed that 47.9% of the total sales were for products with a direct or indirect natural origin. Finally, Cragg and colleagues (Cragg *et al.*, 1997b) showed that of the 520 new drugs in various classes which were approved by the United States Food and Drug Administration from 1983 to 1994, 30 were natural products and 173 were either semi-synthetic based on a natural product core, or modelled on a natural pharmacophore.

Many authors have discussed the importance and potential of medicinal plants as sources of new therapeutic agents (Balandrin *et al.*, 1985; Cordell, 1987, 1995a, 2000; Soejarto and Farnsworth, 1989; Hamburger and Hostettmann, 1991; Cox and Balick, 1994; Lewis and Elvin-Lewis, 1995; Clark, 1996), and this has led to assessments of the value of such plants from a financial perspective (Mendelsohn and Balick, 1995; Principe, 1996). Mendelsohn and Balick (1995) conclude that there are about 375 total drugs of pharmaceutical significance in the rain forests of the world, of which only one-eighth have been discovered. These compounds generate a total value for tropical rain forest pharmaceuticals of $147 billion. Principe has estimated that for OECD countries the value of prescription products from plants likely to go extinct in the next 50 years is $60 million per year (Principe, 1991), and increases as more plants disappear. An alternative view to assigning value to the tropical rainforest is offered by Ehrenfeld (1988), who suggests that 'Assigning value to that which we do not own and whose purpose we do not understand … is the ultimate in presumptious folly'.

Yet, in a continuing, unrelenting process, the temperate and tropical rain forests of the world have been destroyed at a staggering rate over the past 100 years

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(Lugo, 1988; Raven, 1988; Wilson, 1988; Principe, 1991; Anonymous, 1992a,b; McDonald, 1997). Primarily this has occurred because of population increases and the need to clear tracts of land for grazing, for crop cultivation, and for construction timber. Much has been written about these losses from a biodiversity perspective, particularly the taxonomic identification of species of several phyla before they become extinct (Raven, 1988; Wilson, 1988). The latest 'Red List of Threatened Species' issued by the International Union for the Conservation of Nature reports that approximately 34 000 species of flowering plants are threatened in 200 countries (Johnston, 1998). Several authors have also discussed the loss of species which is occurring before adequate (or even any) biological evaluation has taken place (Akerele *et al.*, 1991), and the potential significance of the tropical rainforests as sources of new pharmaceutical agents (Soejarto and Farnsworth, 1989). These arguments have been used to justify protecting the tropical forests (Oldfield, 1989; Abelson, 1990).

From a potential health beneficent perspective, the long-term losses are of the genes for the biosynthesis of the rich chemical diversity in these species; losses which can never be replaced (Ehrlich, 1988). Also being lost prior to adequate documentation is the indigenous knowledge of the shamans, curanderos, hakims, and medicine men and women who are a critical part of the life force in their communities (Balick and Cox, 1996; Cox, 2000). Urbanization and contact with developed groups has frequently drawn apprentices away from acquiring the knowledge of their potential mentors. This places those communities in jeopardy of losing their sustainable, plant-based, health care system in the immediate future. These traditional systems of medicine, including the Traditional Chinese (Tang and Eisenbrand, 1992), the Native American (Vogel, 1990), and the Ayurvedic (Dev, 1999), which have sustained their communities for thousands of years, have provided 74% of the plant-derived pharmaceutical agents (Farnsworth *et al.*, 1985).

We often forget that the approved and regulated chemical armamentarium available today is failing to provide effective health care for even one-third of the human population. In addition, a number of significant global disease states, including cancer, malaria, tuberculosis, and certain viral, fungal and bacterial infections, are showing significant patterns of resistance to the known therapeutic agents (Henry, 2000). Yet most drug discovery efforts aim at the identification of new therapeutic regimens, not at compounds which can overcome drug resistance in various situations.

In the United States, it is generally agreed that the time (10–20 years) and the costs ($450–500 million) to develop a drug from initial discovery to final approval for marketing are both too long and too expensive, respectively. It is also apparent that most newly approved drugs reach the US market only after approval and extensive marketing elsewhere (Mossinghoff, 1992–2000). At the same time, the marketing of unregulated phytotherapeutical products, as dietary supplements, of largely unsubstantiated efficacy, and of undetermined/unreliable consistency and safety has proliferated, both in the retail setting of the pharmacy and the health-food store, and on the internet. The serious scientific concerns regarding these products are only now beginning to be addressed through research programmes sponsored by the United States Government, particularly the National Center for Complementary and Alternative Medicine. In January 2000, the US Food and Drug Administration published a Dietary Supplement Strategy for the next 10 years (Levitt, 2000). However, this strategy still fails to establish reasonable consumer assurances and rigorous scientific standards which, through botanical, chemical and biological standardization, would assure safety and efficacy during the shelf life of a product.

With this brief background, and recognizing and acknowledging what probably lies ahead, it is apparent to these authors that, as a global community of natural product scientists, we must assume a leadership position. We must take the time to examine all of the options, must contemplate very seriously what some of the possibilities for our descendants might be, and must inform our national and international agencies appropriately (Cordell, 1992). We clearly have that responsibility, and must deliberate and take action to the best extent that our intellectual capabilities and extant technology will allow. One can almost hear an echo resounding from the future 'Why didn't they just do something?' Thus, we are graced with a 'window of opportunity', as it has been termed previously (Cordell, 1990a, 1990b, 1993, 1995a, b). This article, and its companion article (Cordell, 2000) is about presenting some ideas of what we can do before that window becomes like the slit window of a medieval castle, narrow, foreboding and extremely limiting of one's vista. This article is also about the potential for human health of a select group of natural products, the alkaloids. These compounds have already demonstrated potent biological activity and profound utility for the health of humankind over thousands of years; highly portentous factors in drug discovery and development.

At the same time we recognize that we are looking from an essentially Western (Northern) perspective of what constitutes a drug and its discovery. Since over 80% of the world's population use plants as their primary source of medication (Farnsworth *et al.*, 1985; Cordell, 2000), our obligation is also to enhance the safety and efficacy of these regimens, recognizing that global access to sophisticated and very expensive Northern drugs is unlikely to ever be achieved. Drug discovery is a rather ethnocentric process, driven as it is by the diseases which dominate the lifestyle of the North, while the drug requirements for the diseases which dominate health care in the South are frequently handled by international organizations such as WHO. As natural product scientists we must attempt to embrace both sets of requirements for the medicinal agents of the future.

Then for whom in the world is drug discovery of relevance? The answer clearly depends on where the question is being asked and by whom. An AIDS victim in Africa or a Karen hill-tribe member in Myanmar will have a quite different perspective to a suburban housewife in Chicago or a bank manager in Tokyo. Perhaps there is greater clarity in asking what will be the critical global diseases of the next 50 years? The many forms of cancer, heart disease, lung disease, HIV infection, Alzheimer's disease and senile dementia, diseases of unknown aetiology, such as arthritis, health conditions that are self-inflicted, such as drug addiction, alcoholism, obesity and smoking, and resistant fungal, bacterial and viral infections would top many lists, to which should probably be added malaria, leishmaniasis, infantile diarrhoea, and tuberculosis. Our challenge is the

discovery and development of the most effective drugs for these diseases; and to do so from a renewable perspective. Thus, we are re-witnessing what our ancestors and many indigenous peoples today know, but which we in the North have forgotten, how to conduct The Discovery of Sustainable Drugs. Biological agents which derive from any renewable resource and/or which are converted to drugs through the use of renewable resources such as multi-functionalized enzyme systems will be important in the years ahead. This vast topic will be the subject of a future presentation.

Why Alkaloids?

This discussion is focused on a group of natural products known as 'alkaloids', and it is certainly appropriate to ask: 'Why alkaloids?'. Why are not flavonoids, lignans, terpenoids or acetogenins discussed in a similar manner? Have they not yielded a range of significant pharmaceutical agents? It is hoped that the following discussion will answer that question and demonstrate the incredible gift that alkaloids are to humankind today. The question of alkaloids in drug discovery will then be placed in light of the current view of desirable drug attributes, and their potential extrapolated by considering how technology is driving the discovery process. Let us begin with an introduction to the discussion, seeking answers to the questions: what is an alkaloid, how many are there, and what are the sources?

The name 'alkaloid' comes from the concept of a compound being 'alkali-like', i.e. basic in character, and containing at least one nitrogen atom. Since many of the initially discovered alkaloids originated from plants, early definitions of an alkaloid included these three characteristics (nitrogen-containing, basicity, and plant origin). As the actual structures and the biogenetic origin of alkaloids became clearer, the concept of being derived from amino acids was added, together with the idea that the nitrogen should be in a heterocyclic ring. A large polypeptide would not be regarded as an alkaloid, however, whereas a small one (<1500 Daltons) would. There were also alkaloids discovered which were neutral or even acidic (e.g. colchicine, quaternary alkaloids), had nitrogen in a chain (e.g. the polyamines), were derived from a purine nucleus (e.g. caffeine), or a preformed acetate or terpenoid precursor with the insertion of nitrogen (e.g. coniine and solanidine, respectively), and these clearly did not fit into any single encompassing definition. Definitions for an alkaloid or a classification are still propounded (Hesse, 1978; Dalton, 1979; Pelletier, 1983; Ikan, 1991; Bruneton, 1993; Torssell, 1997; Roberts and Wink, 1998; Waterman, 1998), but truth to tell there is none that is totally embracing (Cordell, 1981). It is more a case of 'You'll know one when you see one!'

The first alkaloid structure, that of coniine, was elucidated in 1870, and this simple alkaloid was also the first to be successfully synthesized. The number of plant-derived alkaloids characterized was approximately 1000 by 1950, and by 1973 about 3300 structures had been elucidated. Following the dramatic advances in spectroscopic techniques in the past 30 years, a recent analysis of the NAPRALERT^{sm} database† (Farnsworth *et al.*, 1995) indicated 26900 known alkaloid structures from a variety of sources (plants, fungi, marine organisms, mammals, etc.) out of about 150000 characterized natural products.

Although the first alkaloid was isolated from man (spermine phosphate in 1678 by van Leeuwenhoek), the best known sources of alkaloids are plants, fungi, bacteria, and marine animals and microorganisms. More recently, various mammals, vertebrates, parasitic organisms, and insects have served as sources of new alkaloids, and their characterization has substantially expanded the rich chemical diversity of established alkaloid structures. From a biosynthetic perspective, there are relatively few building blocks for alkaloids, and they comprise a selection of common amino acids (ornithine, lysine, phenylalanine/tyrosine, tryptophan, anthranilic acid and histidine), selected polyterpene units, polyketide units and the purines.

Within the plant kingdom, alkaloids are not evenly or ubiquitously distributed. Of the 83 higher plant orders of Cronquist (1981), there are 16 which do not contain alkaloids. According to recent data from the NAPRALERT^{sm} database, alkaloids are distributed in 7231 species of higher plants in 1730 genera (approx. 14.2%) within 186 plant families (Table 1). An additional 35 plant families have alkaloids detected in them, but from which, as yet, no alkaloids have been isolated and characterized (Table 2). There are also 20 plant families (27 genera, 47 species, out of 94 genera, 3751 species) which have been tested in which alkaloids were not detected. There remain 153 plant families (approx. 674 genera and 5835 species) which have never been examined for alkaloids. From a structural diversity perspective, NAPRALERT^{sm} places alkaloids in 27 structural classes (with substructures beneath these); 22 of these alkaloid classes occur in higher plants. A total of 1872 alkaloid skeleta are described, and the distribution of these various alkaloid skeleta types in the monocots, dicots and gymnosperms is shown in Table 3.

The total number of plant-derived alkaloids is 21120, and Table 4 shows the distribution of these alkaloids in the monocots, dicots and gymnosperms. Table 1 indicates the number of alkaloids isolated and characterized from the alkaloid-containing families. On this basis, the 20 most important alkaloid-containing plant families are the Amaryllidaceae, Annonaceae, Apocynaceae, Asteraceae, Berberidaceae, Boraginaceae, Buxaceae, Celastraceae, Fabaceae, Lauraceae, Liliaceae, Loganiaceae, Menispermaceae, Papaveraceae, Piperaceae, Poaceae, Ranunculaceae, Rubiaceae, Rutaceae, and Solanaceae. Table 5 summarizes the 37 plant families from each of which more than 100 different alkaloids have been isolated. An analysis of the highest yielding plant families with more than 50 alkaloids isolated, based on the number of different alkaloids isolated per species in the family, is shown in Table 6. On the other hand, 66 plant families

†**NAPRALERT**^{sm}, an acronym for **NA**tural **PR**oducts **ALERT** is the largest relational database of world literature describing the ethnomedical or traditional uses, chemistry and pharmacology of plant, microbial and animal (primarily marine) extracts. In addition, **NAPRALERT**^{sm} contains considerable data on the chemistry and pharmacology (including human studies) of secondary metabolites of known structure, derived from natural sources. **NAPRALERT**^{sm} currently contains the extracted information from over 150000 scientific research articles dating from 1650 A.D. to the present. These articles contain information on more than 151000 pure chemical species, more than 52000 plant, marine, microbial or animal species, and more than 1.5 million records which associate these records with biological activity.

Table 1. Plant families with isolated alkaloids

	Number of Genera/Species in family[a,b]	Number of Genera/Species alkaloids isolated	Number of alkaloids isolated[c]
Acanthaceae	250/2500	22/35	92
Aceraceae	2/112	1/5	6
Actinidiaceae	3/300	2/3	5
Agavaceae	18/600	1/2	4
Aizoaceae[b]	114/2400	22/65	50
Alangiaceae	1/20	1/9	81
Amaranthaceae	65/900	12/37	39
Amaryllidaceae	??	34/192	448
Anacardiaceae	70/600	8/9	18
Ancistrocladaceae	1/20	1/13	67
Annonaceae	130/2300	59/201	849
Apiaceae	300/3000	32/48	114
Apocynaceae	215/2100	76/400	2664
Aquifoliaceae	4/350	2/19	48
Araceae	110/1800	19/35	75
Araliaceae	70/700	7/9	55
Arecaceae	200/3000	7/7	21
Aristolochiaceae	9/600	5/58	160
Asclepiadaceae	250/3000	19/43	180
Asteraceae	1100/21,000	92/433	705
Basellaceae	4/20	1/2	9
Berberidaceae	13/650	9/138	373
Betulaceae	6/120	5/15	14
Bignoniaceae	110/800	8/11	53
Bombacaceae	30/200	2/2	4
Boraginaceae	100/2000	38/177	287
Brassicaceae	300/3000	35/65	180
Bromeliaceae	45/2000	2/3	10
Buxaceae	5/60	3/15	243
Cactaceae	130/1650	45/138	135
Calycanthaceae[b]	3/9	3/7	10
Campanulaceae	70/2000	7/19	53
Canellaceae	6/20	1/1	3
Cannabidaceae	2/3	2/2	57
Capparidaceae	45/800	17/47	40
Caprifoliaceae	15/400	5/9	15
Caricaceae	4/30	1/3	15
Caryophyllaceae	75/2000	11/12	41
Casuarinaceae	4/70	1/1	1
Celastraceae	50/800	14/50	252
Cephalotaxaceae	1/4	1/9	73
Chenopodiaceae	100/1500	18/53	93
Clusiaceae	50/1200	2/8	6
Combretaceae	20/400	5/9	21
Commelinaceae	50/700	2/2	9
Convolvulaceae	51/1150	22/86	185
Cornaceae	11/100	1/2	1
Crassulaceae	25/900	2/23	47
Cucurbitaceae	90/700	11/19	31
Cupressaceae	17/113	3/4	3
Cycadaceae	1/20	1/1	2
Cynomoriaceae	18/44	2/2	4
Cyperaceae	70/4100	5/9	14
Daphniphyllaceae	1/10	1/6	47
Didymelaceae	1/2	1/2	20
Dilleniaceae	10/350	1/1	1
Dionchophyllaceae	3/3	2/2	25
Dioscoreaceae	6/630	1/12	12
Dipsacaceae	10/270	2/5	5
Ebenaceae	5/450	1/4	5
Elaeagnaceae	3/50	2/6	14
Elaeocarpaceae	10/400	3/14	78
Ephedraceae	1/40	1/33	47
Ericaceae	125/3500	1/3	8
Erythroxylaceae	4/200	1/31	193
Euphorbiaceae	300/7500	33/86	191
Eupomatiaceae	1/2	1/2	14

Table 1. contd.

	Number of Genera/Species in family[a,b]	Number of Genera/Species alkaloids isolated	Number of alkaloids isolated[c]
Fabaceae[b]	657/16,400	174/884	1452
Fagaceae	8/800	3/4	15
Flacourtiaceae	85/800	4/5	21
Garryaceae	1/13	1/3	7
Gentianaceae	75/1000	7/39	25
Geraniaceae	11/700	4/7	16
Gingkoaceae[b]	1/1	1/1	7
Gnetaceae	1/28	1/2	6
Goodeniaceae	14/300	1/1	16
Gyrostemonaceae	5/17	1/1	6
Haemodoraceae	16/100	4/4	2
Hamamelidaceae	26/100	2/2	3
Hernandiaceae	4/60	4/16	146
Himantandraceae	1/3	2/4	12
Hippocastanaceae	2/13	1/2	3
Hippocrateaceae	2/200	3/3	15
Hydrocharitaceae	15/100	2/2	8
Hypoxidaceae	5/100?	3/3	5
Icacinaceae	50/400	5/6	26
Iridaceae	80/1500	2/2	5
Juglandaceae	8/60	2/5	5
Juncaceae	8/200	1/1	2
Lamiaceae	200/3200	29/59	91
Lauraceae	50/2000	25/189	425
Lecythidaceae	20/400	1/1	5
Lemnaceae	6/29	1/1	1
Lentibulariaceae	5/200	1/2	6
Liliaceae	280/4000	45/210	667
Linaceae	13/280	2/4	7
Loasaceae	14/200	3/3	2
Loganiaceae	20/500	7/98	563
Loranthaceae	70/700	7/19	20
Lythraceae	24/500	4/9	53
Magnoliaceae	12/220	9/49	123
Malipighiaceae	60/1200	2/4	22
Malvaceae	75/1200	6/21	48
Meliaceae	51/550	9/21	59
Menispermaceae	70/400	44/164	922
Menyanthaceae	5/33	2/2	4
Monimiaceae	35/450	11/24	115
Moraceae	40/1000	7/16	70
Moringaceae	1/17	1/1	19
Musaceae	2/42	1/4	12
Myristicaceae	15/300	3/14	24
Myrsinaceae	30/1000	3/3	3
Myrtaceae	140/3000	3/3	7
Nyctaginaceae	30/300	3/4	22
Nymphaeaceae	6/54	4/8	76
Nyssaceae	3/8	1/1	30
Ochnaceae	30/400	1/1	4
Olacaceae	30/250	2/3	5
Oleaceae	30/600	6/11	23
Onagraceae	17/675	1/1	1
Orchidaceae	1000/17,800	18/56	53
Orobanchaceae	17/150	3/6	27
Oxalidaceae	8/900	1/1	3
Paeoniaceae	1/30	1/3	5
Pandanaceae	3/725	1/2	6
Papaveraceae[d]	41/660	30/298	1309
Passifloraceae	16/650	1/7	10
Pedaliaceae	20/80	1/1	3
Phytolaccaceae	18/125	4/10	13
Pinaceae[b]	9/194	5/27	56
Piperaceae	10/1940	2/60	258
Plantaginaceae	3/254	1/11	10
Platanaceae	1/7	1/2	3
Plumbaginaceae	12/400	4/9	7

Table 1. contd.

	Number of Genera/Species in family[a,b]	Number of Genera/Species alkaloids isolated	Number of alkaloids isolated[c]
Poaceae	500/8000	36/53	256
Podocarpaceae	12/155	3/5	8
Polygalaceae	12/750	2/3	26
Polygonaceae	30/1000	6/16	32
Pontederiaceae	7/31	1/1	5
Portulacaceae	20/500	1/7	15
Potamogetonaceae	2/90	2/2	2
Primulaceae	30/900	4/4	5
Proteaceae	75/1000	6/10	61
Punicaceae	1/2	1/1	15
Pyrolaceae	4/45	1/1	1
Ranunculaceae	50/2000	22/352	1559
Resedaceae	6/70	1/4	11
Rhamnaceae	55/900	15/45	210
Rhizophoraceae	14/100	6/10	25
Rosaceae	100/3000	21/48	104
Rubiaceae[b]	630/10,400	57/181	677
Rutaceae	150/1500	91/459	1730
Salicaceae	2/340	2/9	9
Salvadoraceae	3/12	2/3	5
Santalaceae	35/400	5/5	13
Sapindaceae	140/1550	5/7	9
Sapotaceae	70/800	6/7	7
Sarraceniaceae	3/15	1/1	1
Saururaceae	5/7	2/4	16
Saxifragaceae	40/700	5/6	27
Scrophulariaceae	190/4000	20/59	106
Simaroubaceae	25/150	15/32	171
Solanaceae	85/2800	37/444	793
Sparganiaceae	1/12	1/1	1
Stemonaceae	3/30	2/8	48
Sterculiaceae	65/1000	9/27	128
Styracaceae	10/50	1/1	1
Symplocaceae	1/300	1/1	2
Taxaceae[b]	6/20	1/2	2
Taxodiaceae[b]	10/14	2/4	16
Theaceae	40/600	1/7	54
Thymelaeaceae	50/500	2/4	11
Tiliaceae	50/450	3/7	10
Trapaceae	1/15	1/1	7
Turneraceae	8/120	1/2	1
Ulmaceae	18/150	2/2	2
Urticaceae	45/700	8/18	23
Valerianaceae	13/300	2/4	16
Verbenaceae	100/2600	8/15	24
Violaceae	16/800	2/3	3
Vitaceae	11/700	4/4	22
Vochysiaceae	7/200	2/2	2
Winteraceae	9/100	1/1	2
Zingiberaceae	51/1150	4/6	12
Zygophyllaceae	30/250	6/19	117

[a] The classification system of Cronquist (1981) is substantially used, with certain exceptions for the alkaloids in the Amaryllidaceae and Hypoxidaceae.
[b] The secondary choice used for the numbers of genera and species in certain families (Aizoaceae, Calycanthaceae, etc.) was Mabberley (1987).
[c] The total number of alkaloids in this column will exceed the total number of known plant-derived alkaloids because of the isolation of the same alkaloid from several different families.
[d] Includes the Fumariaceae.

have less than 10 identified alkaloids, and of those, 30 plant families have three or fewer alkaloids characterized (Table 7).

Plant families frequently have the genetic capacity to produce more than one type of alkaloid. For example, 164 genera in 47 different plant families are capable of producing both isoquinoline (phenylalanine-tyrosine derived) and indole (tryptophan derived) alkaloids. This genetic diversity within plant families is also reflected in the structural diversity of the classes of alkaloid which are isolated. There are 24 plant families which produce ten or more of the 22 NAPRALERT^{sm} structural classes

Table 2. Alkaloid-containing families with no alkaloids isolated

	Genera/Sp. in family[a]	Species tested	Genera (no. tested; no. species in genus[b])
Alismataceae	12/75	4	*Alisma* (2;9), *Echinodorus* (1;47), *Sagittaria* (1;20)
Araucariaceae	2/32	1	*Araucaria* (1;19)
Aspleniaceae	78/2700	1	*Asplenium* (1;650)
Begoniaceae	3/1020	2	*Begonia* (2;900)
Burseraceae	18/600	2	*Boswellia* (1;24), *Commiphora* (1;190)
Chrysobalanaceae	17/450	2	*Moranthes* (1;12), *Paranari* (1;?)
Cistaceae	8/200	3	*Helianthemum* (3;110)
Cneoraceae	1/3	1	*Cneorum* (1;3)
Dichapetalaceae	3/235	1	*Dichapetalum* (1;124)
Elatinaceae	2/40	1	*Bergia* (1;20)
Eucommiaceae	1/1	1	*Eucommia* (1;1)
Fouquieriaceae	1/11	1	*Fouquieria* (1;1)
Gesneriaceae	146/2400	2	*Nautilocalyx* (1;50), *Saintpaulia* (1;20)
Globulariaceae	10/300	1	*Globularia* (1;22)
Hydrophyllaceae	20/250	1	*Hydrolea* (1;20)
Lardizabalaceae	8/30	1	*Akebia* (1;2)
Marantaceae	20/400	2	*Calathea* (1;300), *Maranta* (1;20)
Marattiaceae	7/100	1	*Marattia* (1; 60)
Melastomataceae	200/4000	2	*Melastoma* (1;70), *Memecylon* (1;150)
Melianthaceae	2/8	1	*Melianthus* (1;6)
Molluginaceae	13/100	2	*Mollugo* (2;15)
Myoporaceae	4/125	1	*Myoporum* (1;32)
Myricaceae	3/50	2	*Comptonia* (1;1), *Myrica* (1;50)
Nolanaceae	2/66	1	*Nolana* (1;18)
Opiliaceae	9/50	4	*Cansjera* (1;2), *Opilia* (1;2), *Rhopalopilia* (2;4)
Pandaceae	3/15	2	*Microdesmis* (1;10), *Panda* (1;1)
Pittosporaceae	9/240	1	*Pittosporum* (1;200)
Polemoniaceae	18/300	4	*Cobaea* (1;1), *Leptodactylon* (1;12), *Phlox* (2;67)
Polypodiaceae	52/550	1	*Polypodium* (1; 75)
Schisandraceae	2/50	1	*Schisandra* (1;25)
Staphyleaceae	5/50	1	*Turpinia* (1;10)
Taccaceae	1/10	1	*Tacca* (1;1)
Tamaricaceae	5/100	5	*Reaumuria* (2;11), *Tamarix* (3;54)
Typhaceae	1/10	3	*Typha* (1;10)
Zamiaceae	8/100	1	*Zamia* (1;40)

[a] The classification system of Cronquist (1981) is substantially used, with certain exceptions for the families Aspleniaceae, Marattiaceae, and Zamiaceae for which Mabberley (1987) was used.
[b] Data are from Mabberley (1987).

of alkaloid, and the number of alkaloids in these structure classes which are produced by these 24 plant families are shown in Table 8.

Alkaloids are also not evenly distributed within a particular plant. Some alkaloids are concentrated in the roots (reserpine), while others may be located predominantly in the leaves (nicotine), the fruits (strychnine), the bark (quinine) or the latex (morphine). More recently it has become apparent that certainly in plants, the biosynthetic apparatus for the formation of alkaloids is frequently located in more than one site in plant cells (Kutchan, 1998; Verpoorte *et al.*, 1998; Wink and Roberts, 1998). This information is of substantial significance as consideration is given as to what plant parts to collect for alkaloid extraction, and how the genetic apparatus of alkaloid biosynthesis may be discerned and possibly relocated to other systems (Verpoorte *et al.*, 1998).

Alkaloids in the service of humankind

Man's intimate, albeit unaware, relationship with alkaloids dates back to the earliest known Sumerian clay tablets which described the medicinal plants being used at that time. Furthermore, we are aware that the use of alkaloid-containing beverages as stimulants (e.g. tea and coffee) is very old. Cultivation of tea, for example, probably dates back to the 12th century B.C.E. in Sichuan Province in China, where cultivation continues today. The legend of coffee, 'Qahwah', begins in Ethiopia, or may be derived from indigenous groups in Central Africa who used the stimulant properties of coffee beans during long treks (Quimme, 1976). Other alkaloid-based stimulants (khat, betel nut, etc.) may date back much further in time.

For all of us, this intimate relationship with many alkaloids continues on a day-to-day basis; there are those we love and those which we fear. In the latter category there are the arrow poisons derived from curare and which contain potent muscle relaxants, such as C-toxiferine and tubocurarine. There are the animal toxins derived from the puffer fish (tetrodotoxin) (Mosher, 1986), and from the *Dendrobates* and *Phyllobates* frogs (batrachotoxins, pumiliotoxins and histrionicotoxins) (Daly *et al.*, 1993, 1997), and there are the toxins from the marine 'red tides', such as saxitoxin (Mosher, 1986).

Table 3. Distribution of alkaloid skeleta in monocots, dicots and gymnosperms

Alkaloid class	Monocots	Dicots	Gymnosperms	Total
Simple amines	17	48	6	71
Pyrrolidine/piperidine	19	51	4	74
Pyridine	3	33	1	37
Tropane	2	13	1	16
Pyrrolizidine	11	20	0	31
Indolizidine	4	21	0	25
Quinolizidine	3	45	0	48
Quinoline	3	125	3	131
Isoquinoline	71	287	14	372
Indole	30	539	2	571
Monoterpene	1	23	0	24
Sesquiterpene	5	30	0	35
Diterpene	2	73	3	78
Triterpene	1	29	0	30
Steroidal	32	55	0	87
Peptide	1	20	0	21
Spermine/spermidine	2	12	5	19
Flavonoid alkaloids	1	5	0	6
Misc. one N	29	62	0	91
Misc. two N	16	55	3	73
Misc. three N	1	11	0	12
Misc. four N	4	15	1	20
Total	258	1571	43	1872

From many wide-ranging plants in the Leguminosae and Crotalariaceae there are the hepatotoxic 1,2-dehydropyrrolizidine alkaloids (Mattocks, 1986), and then there are those alkaloids which produce very dramatic teratogenic effects, such as cyclopamine, present in grazing plants such as *Veratrum californicum* Durand (Keeler *et al.*, 1991; Gaffield, 2000). Finally, there are the toxic mushrooms whose activity is often traced to alkaloids (Hatfield, 1979).

Many alkaloids are known to have the ability to change the perception of reality, including those of *Datura, Psilocybe*, peyote and ergot. There are the euphoric alkaloids of opium, and the devastating alkaloids of ergot responsible for epidemics of vasoconstriction (St Anthony's Fire) and hallucinations in the Middle Ages in Europe. There is also the acetylcholinesterase (ACE) inhibitor present in the Calabar Bean (*Physostigma venenosum* Balf., Fabaceae, physostigmine) and the

Table 4. Distribution of alkaloids in monocots, dicots and gymnosperms by structure type

Alkaloid class	Monocots	Dicots	Gymnosperms	Total[a]
Simple amines	98	1141	20	1213
Pyrrolidine/piperidine	126	773	32	898
Pyridine	22	299	5	314
Tropane	6	495	4	502
Pyrrolizidine	40	855	–	884
Indolizidine	5	240	–	245
Quinolizidine	5	768	–	771
Quinoline	7	1175	6	1184
Isoquinoline	739	5040	175	5872
Indole	142	5112	6	5191
Monoterpene	1	132	–	132
Sesquiterpene	12	214	–	226
Diterpene	4	1302	3	1307
Triterpene	1	122	–	123
Steroidal	329	547	–	849
Peptide	2	160	–	161
Spermine/spermidine	8	217	8	221
Flavonoid alkaloids	1	18	–	19
Misc. one N	143	236	–	366
Misc. two N	52	386	4	411
Misc. three N	2	14	–	15
Misc. four N	29	203	12	216
Total	1774	19 449	275	21 120

[a] Alkaloid numbers do not sum horizontally in cases where the same alkaloid has been isolated from monocots, dicots and gymnosperms.

Table 5. Plant families with more than 100 alkaloids isolated

Plant family	Number of alkaloids	Plant family	Number of alkaloids
Apocynaceae	2664	Buxaceae	243
Rutaceae	1730	Rhamnaceae	210
Ranunculaceae	1559	Erythroxylaceae	193
Fabaceae	1453	Euphorbiaceae	191
Papaveraceae	1309	Convolvulaceae	185
Menispermaceae	922	Asclepiadaceae	180
Annonaceae	849	Brassicaceae	180
Solanaceae	793	Simaroubaceae	171
Asteraceae	705	Aristolochiaceae	160
Rubiaceae	677	Hernandiaceae	146
Liliaceae	667	Cactaceae	135
Loganiaceae	563	Sterculiaceae	128
Amaryllidaceae	448	Magnoliaceae	123
Lauraceae	425	Zygophyllaceae	117
Berberidaceae	373	Monimiaceae	115
Boraginaceae	287	Apiaceae	114
Piperaceae	258	Scrophulariaceae	106
Poaceae	256	Rosaceae	104
Celastraceae	252		

parasympathomimetic in Jaborandi (*Pilocarpus jaborandi* Holmes, Rutaceae, pilocarpine), whose activities were used in the ritual ceremonies of indigenous groups for truth-telling.

And what of the alkaloids we love (not always voluntarily)? Many of us appreciate the profound stimulant effects of the caffeine found in coffee, tea, guarana and maté, and the theobromine in chocolate. Many cuisines in the world use, as a critical spice, various peppers whose active principle is either capsaicin or the

Table 6. Highest yielding alkaloid-containing families by species[a]

Family	Number of species studied	Number of isolated alkaloids	Number of alkaloids/ species studied	Number of species to be studied[b]
Cannabidaceae	2	58	29.0	1
Buxaceae	15	243	16.2	45
Nymphaeaceae	8	76	9.50	52
Hernandiaceae	16	146	9.13	52
Alangiaceae	9	81	9.00	8
Theaceae	7	54	7.71	513
Apocynaceae	400	2664	6.66	1700
Zygophyllaceae	19	119	6.26	241
Erythroxylaceae	31	193	6.23	229
Araliaceae	9	55	6.11	791
Proteaceae	10	61	6.10	1340
Lythraceae	9	53	5.89	571
Loganiaceae	98	567	5.79	502
Menispermaceae	164	922	5.62	356
Elaeocarpaceae	14	78	5.57	506
Simaroubaceae	32	171	5.34	138
Ancistrocladaceae	13	67	5.15	–
Celastraceae	50	253	5.06	1250
Monimiaceae	24	116	4.83	426
Poaceae	53	256	4.83	8947
Bignoniaceae	11	53	4.82	714
Sterculiaceae	27	128	4.74	1473
Rhamnaceae	45	210	4.67	830
Ranunculaceae	352	1559	4.43	1398
Papaveraceae[c]	298	1309	4.39	660
Moraceae	16	70	4.38	1184
Piperaceae	60	258	4.30	1880
Annonaceae	201	849	4.22	1849
Asclepiadaceae	43	180	4.19	2803

[a] Number of different alkaloids per species based on alkaloid families with more than 50 alkaloids isolated. Only alkaloid-containing families with more than 4 alkaloids isolated per species studied are listed.
[b] Data are from Mabberley (1987).
[c] Includes Fumariaceae.

Table 7. Plant families with three or fewer alkaloids isolated

Family	Number of alkaloids	Alkaloid type(s) (number of alks)
Buddlejaceae	2	Piperidine/pyrrolidine; Misc. one N
Canellaceae	3	Isoquinoline (1); Indole (2)
Casuarinaceae	1	Pyrrolizidine
Cornaceae	1	Isoquinoline
Cupressaceae	3	Simple amines (2); Isoquinoline (1)
Cycadaceae	2	Simple amines
Dilleniaceae	1	Misc. four N
Haemodoraceae	2	Isoquinoline
Hamamelidaceae	3	Simple amines
Hippocastanaceae	3	Simple amines (2); Misc. two N (1)
Juncaceae	2	Simple amine; Quinolizidine
Lemnaceae	1	Simple amine
Loasaceae	2	Simple amine; Indole
Myrsinaceae	3	Simple amines (2); Misc. four N (1)
Onagraceae	1	Indole
Oxalidaceae	3	Simple amines (1); Pyridine (1); Quinoline (1)
Pedaliaceae	3	Monoterpene (3)
Platanaceae	3	Misc. two N (1); Misc. four N (2)
Potamogetonaceae	2	Misc. one N; Misc. four N
Pyrolaceae	1	Simple amine
Sarraceniaceae	1	Piperidine/pyrrolidine
Sparganiaceae	1	Piperidine/pyrrolidine
Styracaceae	1	Quinoline
Symplocaceae	2	Isoquinoline
Taxaceae	2	Diterpene
Turneraceae	1	Misc. four N
Ulmaceae	2	Pyridine; Isoquinoline
Violaceae	2	Simple amine (1); Isoquinoline (2)
Vochysiaceae	2	Simple amine; flavonoid alkaloid
Winteraceae	2	Indolizidine

Murraya alkaloids. Enormous numbers of peoples all over the world are now dependent on tobacco products, and the alkaloid nicotine (Goodman, 1994). Three other of the world's major dependence producing stimulant agents are also alkaloid based, namely, coca, khat and betel nut. In addition, opium and its 3,6-*O*-diacetyl morphine derivative, heroin, remain major items of illicit commerce.

What have been some of the positive contributions of alkaloids to global society thus far? We will examine these contributions in terms of health care, biology and organic chemistry, and also make mention of the alkaloid which probably saved western civilization as we know it.

The first extended studies on the isolation of alkaloids by Pelletier and Caventou at the University of Paris in the period from 1819 were based on the well-established, medicinal or biological uses of the plants *Strychnos nux vomica* L., *Cephaelis ipecacuanha* (Brotero) A. Richard, *Piper nigrum* L., *Coffea arabica* L., *Cinchona succirubra* Pavon ex Klotzsch, *Colchicum autumnale* L. and *Conium maculatum* L. Since that time, numerous other plants used ethnomedically for a wide variety of disease states have been examined and, in many instances, their active principles were determined to be alkaloids. Morphine, the first commercial natural product (Newman *et al.*, 2000) was first marketed in 1826. By the late 20th century, of the 119 compounds from 90 plants that were used as single entity medicinal agents, 54 were alkaloids (Farnsworth *et al.*, 1985). Schmeller and Wink (1998) have described the origins, therapeutic use and mechanism of action of 44 alkaloids. A reassessment of the number of alkaloids of pharmaceutical and biological significance globally indicates that this contribution by alkaloids continues to increase steadily and now numbers about 60 agents, exclusive of the ergot alkaloids (Table 9). Some representative examples are shown in Fig. 1. From an ethnomedical perspective, 39 (65.0%) of these agents are used pharmacologically in a manner which correlates with their indigenous use. There are also several alkaloid derivatives which are used as pharmaceutical agents (Cragg *et al.*, 1997b; De Smet, 1997; Grifo *et al.*, 1997; Newman *et al.*, 2000). In addition, there are many pharmaceutical agents whose molecular phylogeny indicates that they contain critical pharmacophoric elements from an alkaloid, such as fendiline (based on atropine), atracurium (based on tubocurarine) and neostigmine (based on physostigmine) (Foye, 1995).

We have seen that the success of alkaloids as the basis for pharmacotherapy is largely based on substantial historical experience with the parent plant. In considering the potential of alkaloids for drug discovery and development for the future it is important to ask whether, as biology has developed to become more molecularly based in the past 30 years, have alkaloids continued to provide important biological agents? The answer is clearly, 'Yes'.

Several alkaloids have served, and continue to serve, as important tools in the elucidation of key pharmacological effects, physiological responses and biochemical mechanisms. Alkaloids have been important in the discovery and development of the operation and functioning of receptors and ion channels. The important, multi-faceted ecological role of various alkaloids has led to significant new awarenesses of our relationship with the biome and of the interrelationships between species (Wink, 1998).

Table 8. Plant families with 10 or more types of alkaloids

	Alkaloid type																					
Family	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	Total
Acanthaceae	8	5	2	1	0	2	0	4	1	10	0	0	0	0	0	0	7	0	4	48	0	2	92
Annonaceae	18	5	7	0	0	0	1	6	662	85	0	7	0	0	0	0	0	1	49	5	1	2	849
Apiaceae	33	21	7	0	1	0	1	1	9	11	0	0	0	0	0	0	2	0	0	5	0	23	114
Apocynaceae	11	1	1	8	22	0	2	27	11	2431	13	5	0	2	93	0	11	0	0	6	0	20	2664
Araliaceae	3	2	1	0	0	1	1	0	6	15	0	0	0	0	0	0	2	0	0	13	1	10	55
Asclepiadaceae	7	0	5	0	0	101	1	2	8	27	0	0	0	0	13	0	0	0	0	7	0	9	180
Asteraceae	169	90	4	1	320	0	4	10	10	37	0	3	6	0	0	6	2	0	1	15	0	27	705
Brassicaceae	62	9	3	1	0	0	2	3	5	64	0	0	0	0	0	0	0	0	0	16	3	12	180
Celastraceae	8	3	1	0	2	0	0	3	27	4	0	166	1	0	0	3	27	0	1	2	0	4	252
Convolvulaceae	8	11	1	38	55	9	10	0	4	37	0	0	0	0	1	1	0	0	0	1	0	9	185
Euphorbiaceae	18	33	5	2	6	20	2	3	59	9	0	0	1	0	0	4	0	0	13	4	2	10	191
Fabaceae	111	107	26	2	110	21	603	6	160	100	2	1	53	0	1	3	38	4	1	68	1	35	1453
Lamiaceae	15	7	37	0	3	0	1	4	0	6	1	0	1	0	10	0	0	0	1	1	0	4	91
Lauraceae	7	4	4	0	0	7	2	0	390	9	0	1	0	0	0	0	0	0	1	0	0	0	425
Liliaceae	16	60	1	0	5	1	2	0	242	13	0	0	1	0	313	0	0	1	1	5	0	6	667
Moraceae	4	33	6	3	1	10	0	5	2	1	0	0	0	0	0	0	0	2	0	0	0	4	71
Orchidaceae	2	5	2	0	20	2	0	0	5	8	0	5	0	0	0	0	0	0	0	2	0	2	53
Papaveraceae	18	3	2	0	1	0	1	1	1256	15	0	0	0	0	0	0	0	0	7	2	0	4	1310
Poaceae	53	12	14	0	14	0	2	7	16	92	0	1	0	0	0	2	2	0	0	27	0	14	256
Ranunculaceae	26	0	2	0	2	0	3	3	489	12	0	0	1011	0	2	0	0	0	2	2	0	5	1559
Rubiaceae	21	38	12	0	0	0	1	70	44	391	11	0	0	3	0	3	0	0	13	54	0	16	677
Rutaceae	138	13	35	0	1	0	2	847	283	369	2	0	1	0	0	0	1	0	4	27	1	6	1730
Scrophulariaceae	8	5	3	0	8	0	21	0	0	3	15	0	0	0	0	0	31	0	0	8	0	4	106
Solanaceae	79	54	101	204	1	1	2	5	27	18	2	0	0	0	230	0	13	0	0	25	2	29	793
Total	843	521	282	260	572	175	664	1007	3716	3767	46	189	1075	5	663	22	136	8	98	341	11	257	14658

Alkaloid structure types: A, Simple amines; B, Piperidine/pyrrolidine; C, Pyridine; D, Tropane; E, Pyrrolizidine; F, Indolizidine; G, Quinolizidine; H, Quinoline; I, Isoquinoline; J, Indole; K, Monoterpene; L, Sesquiterpene; M, Diterpene; N, Triterpene; O, Steroidal; P, Peptide; Q, Spermine/Spermidine; R, Flavonoid Alkaloids; S, Misc. One N; T, Misc. Two N; U, Misc. Three N; V, Misc. Four N.

Table 9. Plant-derived alkaloids of pharmaceutical and biological significance[a]

Alkaloid[b]	Source	Family	Pharmaceutical/biological use
Aconitine	*Aconitum napellus*	Ranunculaceae	Rheumatism, neuralgia
Ajmalicine*	*Catharanthus roseus*	Apocynaceae	Circulatory disorders
Ajmaline	*Rauvolfia serpentina*	Apocynaceae	Cardiac arrythmia
Anabasine	*Anabasis aphylla*	Fabaceae	Skeletal muscle relaxant
Anisodine*	*Scopolia tanguticus*	Solanaceae	Anticholinergic
Arecoline*	*Areca catechu*	Palmae	Anthelmintic
Atropine*	*Atropa belladonna*	Solanaceae	Antispasmodic, mydriatic
	Duboisia myoporoides	Solanaceae	
Berberine*	*Berberis vulgaris*	Berberidaceae	Bacillary dysentry, eye infections
Boldine*	*Peumus boldo*	Monimiaceae	Anti-cholelithiasis, stomachic
Caffeine*	*Camelia sinensis,*	Theaceae	CNS stimulant
	Coffea arabica	Rubiaceae	
Camptothecin*	*Camptotheca acuminata*	Icacinaceae	Antitumour
Capsaicin	*Capsicum annuum*	Solanceae	Peripheral neuralgia
Cocaine*	*Erythroxylum coca*	Erythroxylaceae	Local anaesthetic
Codeine*	*Papaver somniferum*	Papaveraceae	Analgesic; antitussive
Colchicine*	*Colchicum autumnale,*	Liliaceae	Anti-gout, amyloidosis
	Gloriosa superba	Liliaceae	
Conessine*	*Holarrhena antidysenterica*	Apocynaceae	Antidysenteric
Deserpidine*	*Rauvolfia canescens*	Apocynaceae	Antihypertensive
L-Dopa	*Mucuna derringiana*	Fabaceae	Anti-Parkinsonism
Ellipticine	*Ochrosia elliptica*	Apocynaceae	Antitumour
Emetine*	*Cephaelis ipecacuanha*	Rubiaceae	Amoebicide; emetic
Ephedrine*	*Ephedra sinica*	Ephedraceae	Decongestant, antiasthmatic
Galanthamine	*Galanthus woronowii*	Amaryllidaceae	Cholinesterase inhibitor
Glaucine	*Glaucium flavum*	Papaveraceae	Antitussive
Glaziovine	*Ocotea glaziovii*	Lauraceae	Antidepressant
Homoharringtonine*	*Cephalotaxus harringtonia*	Cephalotaxaceae	Antitumour
Huperzine A*	*Huperzia serrata*	Lycopodiaceae	Senile dementia
β-Hydrastine*	*Hydrastis canadensis*	Ranunculaceae	Astringent; G I disorders
Hyoscyamine*	*Hyoscyamus niger*	Solanaceae	Anticholinergic
Lobeline*	*Lobelia inflata*	Campanulaceae	Smoking deterrent
Melatonin	*Avena sativa*	Poaceae	Muscle relaxant
Monocrotaline*	*Crotalaria sessiliflora*	Fabaceae	Antitumour
Morphine*	*Papaver somniferum*	Papaveraceae	Analgesic
Narceine*	*Papaver somniferum*	Papaveraceae	Antitussive
Nicotine	*Nicotiana tabacum*	Solanaceae	Insecticide
Noscapine*	*Papaver somniferum*	Papaveraceae	Antitussive
Palmatine	*Coptis japonica*	Berberidaceae	Antipyretic
Papaverine	*Papaver somniferum*	Papaveraceae	Smooth muscle relaxant
Physostigmine*	*Physostigma venenosum*	Fabaceae	Cholinesterase inhibitor
Pilocarpine*	*Pilocarpus jaborandi*	Rutaceae	Parasympathomimetic
Pseudoephedrine*	*Ephedra sinica*	Ephedraceae	Sympathomimetic
Pseudoephedrine, nor*	*Catha edulis*	Celastraceae	Sympathomimetic
Quinidine	*Cinchona ledgeriana*	Rubiaceae	Antiarrhythmic
Quinine*	*Cinchona ledgeriana*	Rubiaceae	Antimalarial; tonic
Rescinnamine*	*Rauvolfia serpentina*	Apocynaceae	Antihypertensive; tranquillizer
Reserpine*	*Rauvolfia serpentina*	Apocynaceae	Antihypertensive; tranquillizer
Sanguinarine	*Sanguinaria canadensis*	Papaveraceae	Dental plaque inhibitor
Scopolamine*	*Datura metel*	Solanaceae	Sedative; motion sickness
Sparteine	*Cytisus scoparius*	Fabaceae	Oxytocic; cardiac arrythmias
Strychnine*	*Styrchnos nux-vomica*	Loganiaceae	CNS stimulant
Taxol	*Taxus brevifolia*	Taxaceae	Antitumour
Tetrahydropalmatine*	*Stephania sinica*	Amaryllidaceae	Analgesic; sedative
	Corydalis ambigua	Papaveraceae	
Tetrandrine	*Stephania tetrandra*	Amaryllidaceae	Antihypertensive
Theobromine*	*Theobroma cacao*	Sterculiaceae	Diuretic; vasodilator
Theophylline*	*Camellia sinensis*	Theaceae	Diuretic; bronchodilator
Tubocurarine*	*Chondrodendron tomentosum*	Menispermaceae	Skeletal muscle relaxant
Vasicine (Peganine)	*Adhatoda vasica*	Acanthaceae	Oxytocic
Vinblastine	*Catharanthus roseus*	Apocynaceae	Antitumour
Vincamine*	*Vinca minor*	Apocynaceae	Cerebral stimulant
Vincristine	*Catharanthus roseus*	Apocynaceae	Antitumour
Yohimbine*	*Pausinystalia yohimba*	Rubiaceae	Aphrodisiac

[a] Modified and updated from Farnsworth *et al.* (1985).
[b] Ajmalicine is also known as raubasine; atropine is racemic hyoscyamine; noscapine is also known as narcotine; norpseudoephedrine is also known as cathine; physostigmine is also known as eserine; scopolamine is also known as hyoscine.
* Indicates that the pharmaceutical use correlates with the ethnomedical use.

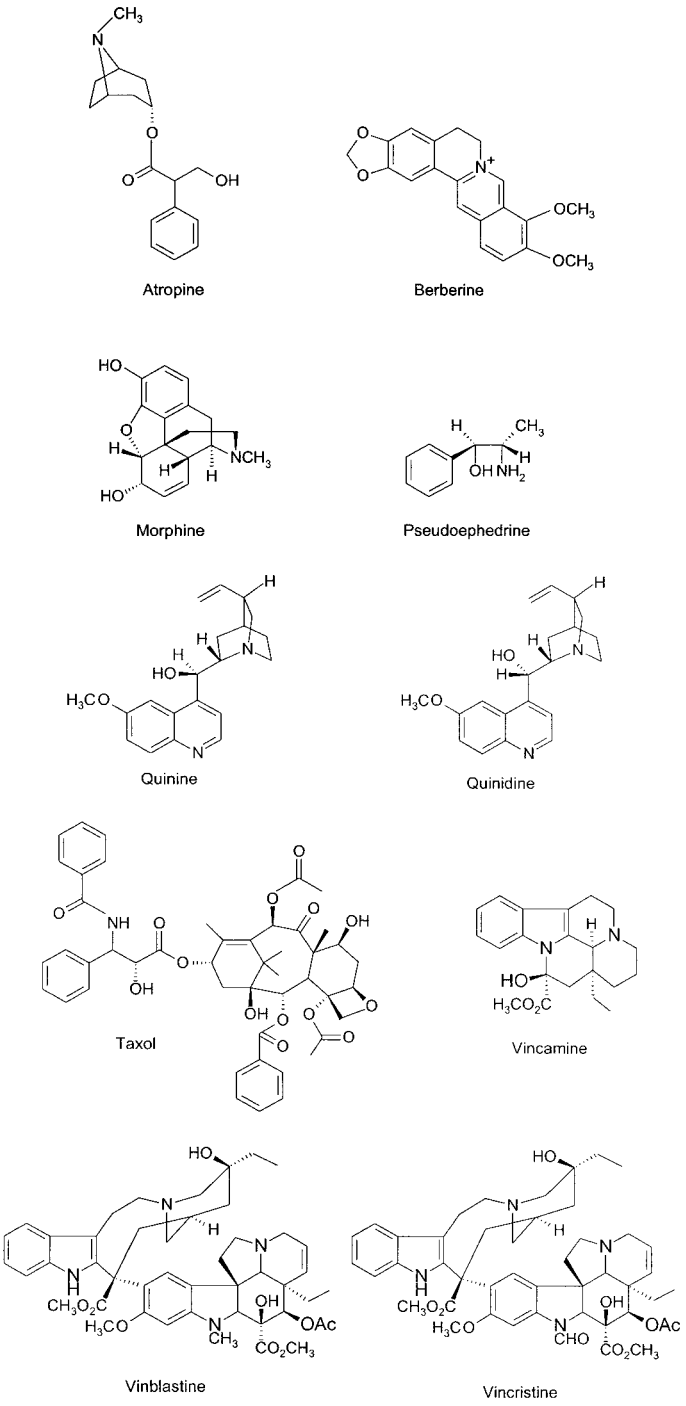

Figure 1. A selection of alkaloids of pharmaceutical importance.

Wink has provided an excellent and extensive summary of the actions of alkaloids (their interference) with numerous receptors and ion channels (Wink, 2000). Data are presented for alkaloids which are agonists/antagonists at the nicotinic acetylcholine receptor, the muscarinic acetylcholine receptor, the alpha and beta adrenergic receptors, the serotonin receptors, the dopamine receptors, GABA receptors, and the glutamate and opiate receptors. The alkaloids which affect sodium, potassium, and calcium channels are summarized, together with alkaloids possessing acetylcholinesterase inhibiting activity, and those which inhibit neurotransmitter uptake. Those alkaloids binding to DNA and which affect protein synthesis are also summarized. It is pointed out that there are several very potent alkaloids known for most of these receptors (sometimes the most active compound known is an alkaloid), that alkaloids of diverse structure are active for a particular receptor, and that several alkaloids are active against more than one molecular target. Wink interprets this in terms of a multipurpose defence function (vs herbivores and bacterial and fungal infestation) which has developed during evolutionary time. He suggests that this diverse activity implies that other, less dominant, alkaloids in the plant will have reduced and/or more selective activity for certain receptors. The summarized information also demonstrates that the response data in these basic biological systems are not available, even for the

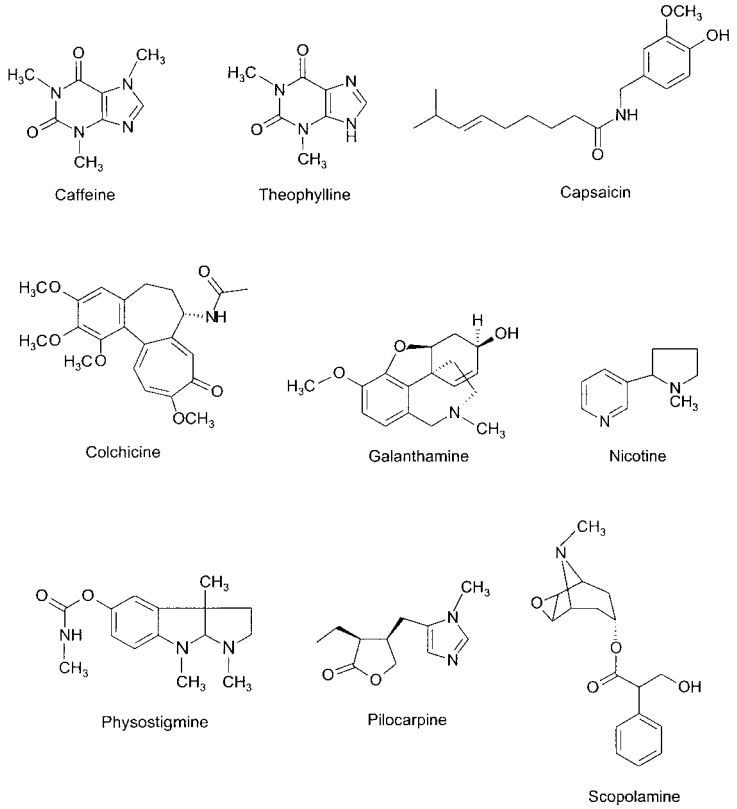

Figure 2. Some old alkaloids with new applications.

evaluation of many of the 'parent' alkaloids (Wink, 2000). This aspect of the biological testing of alkaloids will be touched on again subsequently.

Over the years, many have pondered the role and function of alkaloids in their host organism. Although once regarded as waste products of metabolism, this is now clearly established not to be the case. The interactions between alkaloid-containing plants and various herbivores is deep and complex (Wink, 1998). From feeding deterrent and insecticidal to being acquired by an invertebrate as a chemical defence to antifungal, antibacterial and antiviral activity against invading organisms, alkaloids have a significant ecological niche. In addition, several alkaloids have been shown to serve as the sole carbon and nitrogen source for bacteria (Lister *et al.*, 2001).

One aspect of the dependent, evolutionary relationship between drug discovery, plants and other living creatures in the rain forest is demonstrated by the structurally diverse, biologically potent alkaloids which occur in frog skin (Daly *et al.*, 1993; Daly, 1998). These alkaloids have proved to be powerful biological tools in studying pharmacological responses at the molecular level. However, the diet of these frogs in captivity does not typically yield the desired alkaloids present in the skins of native frogs. Thus, the habitat of the flora and arthropods which are a part of the food chain is critical for alkaloid synthesis and sequestration (Daly *et al.*, 1997). As the rainforests disappear, we are losing these habitats, frog species are disappearing (Anon, 1999), and with them additional novel biological agents.

From a chemical perspective, the study of alkaloids has advanced organic chemistry in three significant areas: organic synthesis, drug development and chiral synthesis. Many of the classical target molecules of organic synthesis that were developed in the 1940s to 1980s were alkaloids. In this regard, one thinks of the classic efforts of Woodward in developing stereospecific syntheses of reserpine, and strychnine, among others (Bindra and Bindra, 1975), and the strategies of Barton for the formation of benzylisoquinoline-derived alkaloids through phenol oxidative coupling (Barton, 1963; Barton *et al.*, 1966). In the area of drug development, many of the synthetic drugs that are available today have a phylogeny which can be traced to alkaloids such as papaverine or atropine (Foye, 1995). More recently, selected alkaloids, particularly quinine/dihydroquinine and sparteine, have contributed substantially as catalysts in the development of several types of enantiomeric, stereospecific synthesis (Song, 2000).

And what of the alkaloid that saved western civilization? For a stimulating discussion of this assertion one only has to read a summary of the exciting history of quinine during the latter part of the Second World War, and the development of chloroquine in *Plants, People and Culture. The Science of Ethnobotany* (Balick and Cox, 1996).

Alkaloids of Current Interest

Many of the alkaloids that are in therapeutic use are regarded as 'old' compounds. Yet, as biological evaluation of these compounds continues, unexpected uses are being disclosed. De Smet (1997) has discussed some of the new biological applications for these 'old' alkaloids, such as caffeine (neonatal apnoea), capsaicin (postherpe-

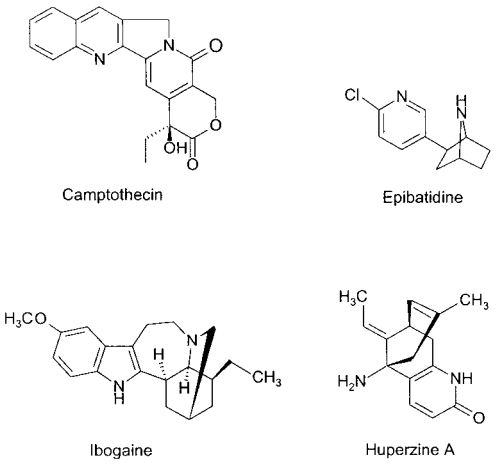

Figure 3. Some alkaloids of current interest.

tic neuralgia, *vide infra*), colchicine (familial Mediterranean fever), galanthamine (Alzheimer's disease), nicotine (ulcerative colitis, Tourette's syndrome), physostigmine (Alzheimer's disease), pilocarpine (xerostomia), quinine (nocturnal leg cramps), scopolamine (nausea and vomiting), and theophylline (anti-inflammatory)(Fig. 2). In spite of the absence of targeted programmes to investigate alkaloids, a number of alkaloids have recently been introduced into the market and/or have sparked intense chemical and biological interest. Space permits only a selection of these to be discussed here (Fig. 3), thus taxol (paclitaxel) (Suffness, 1995), and three anticancer agents, pancratistatin (Pettit *et al.*, 1995), homoharringtonine (Hehlmann *et al.*, 2000) and indirubin (Buolamwini, 2000), which are in advanced stages of development at the National Cancer Institute, are not discussed.

Camptothecin is a monoterpene indole alkaloid isolated from the Chinese tree *Camptotheca acuminata* Decne. (Icacinaceae) which displays potent antitumour activity (Wall *et al.*, 1966; Potmesil and Pinedo, 1995). What sparked renewed interest in developing camptothecin as a drug was the discovery that the mechanism of action, interference with topoisomerase I, was novel (Hsiang *et al.*, 1985; Hsiang and Liu, 1988). Because of the lipophilicity of camptothecin, substantial efforts have been made to develop analogues with enhanced water solubility or pharmacokinetics. Two such derivatives, irinotecan and topotecan are currently widely available, and other derivatives are in advanced clinical trials or earlier stages of development.

The Ecuadorean rain forest frog *Epipedobates tricolor* yielded minute quantities of epibatidine (Daly *et al.*, 1978), whose analgesic mechanism of action as a potent nicotinic agonist was quite distinct from that of the opiates, since it had a low affinity for opioid receptors (Spande *et al.*, 1992; Badio *et al.*, 1994; Daly *et al.*, 2000). The relative simplicity of the alkaloid led to the development of a number of syntheses which confirmed the structure and also allowed the exploration of structure–activity relationships (Szántay 1995). ABT-594, from Abbott Pharmaceuticals, is an analogue which has similar affinity for the nicotine subtype as epibatidine in the CNS, but shows 4000 times less affinity at the neuromuscular junction, thereby potentially reducing *in vivo* side effects (Bannon *et al.*, 1998).

The indole alkaloid ibogaine, first isolated from the root bark of the West African tree *Tabernanthe iboga* Baillon (Apocynaceae) in 1901, is an alkaloid of substantial interest at the present (Alper, 2001). The ethnomedical use of the plant relates to initiation into adulthood in the Bwiti religion (Fernandez and Fernandez, 1990, 2001), and efforts are currently underway to examine the potential of ibogaine to act as an anti-addictive agent for heroin, cocaine and alcohol (Alper, 2001). Substantial mechanistic work has already demonstrated that ibogaine acts at multiple sites, and this has led to the concept that in order to develop effective antiaddictive regimens, agents (or a group of agents) which act through a diversity of mechanisms should be used clinically (Alper, 2001). The high selectivity of ibogaine for sigma-2 receptors, unique among all compounds, indicates once again that alkaloids have a very high potential to be significant pharmaceutical agents. Indeed, ibogaine may well provide new paradigms for understanding the neurobiology of drug addiction.

Although not a higher plant, the club moss *Huperzia serrata* (Thunb.) Trev. (syn. *Lycopodium serratum* Thunb.) known as Qian Ceng Ta in Traditional Chinese Medicine, which is used for the treatment of fever and inflammation, has yielded a number of related, novel alkaloids called huperzines (Liu *et al.*, 1986). The principal alkaloid, huperzine A, is a potent, reversible acetylcholinesterase (ACE) inhibitor which binds more tightly to the receptor than tacrine or donepezil and may have fewer side effects. Some synthetic analogues (e.g. the 10α-methyl derivative) have shown higher levels of activity (Kozikowski *et al.*, 1996; Skolnick, 1997). The alkaloid is already available in the United States in stores and through the internet for 'improving cognitive function' following clinical trials for memory enhancement and cognition in China (Borman, 1998a).

The unsaturated fatty acid amide capsaicin is one of the 'hot' principles of chili peppers (*Capsicum* spp.) in culinary use in many parts of the world. Further pharmacological studies revealed that it depletes and prevents the reaccumulation of substance P, which is an important mediator of peripheral pain to the CNS (Rumsfeld and West, 1991; Cordell and Araujo, 1993). As such it is recommended as a topical medication for the treatment of postherpetic neuralgia due to osteoarthritis or diabetic neuropathy.

The Potential of Alkaloids in Lead Discovery

The American philosopher Ralph Waldo Emerson (1803–1882), in an essay on Nature, wrote 'What is a weed? A plant whose virtues have not yet been discovered'. All lead compound discovery is based on creating value in chemical entities which initially have no intrinsic value. If that chemical entity is derived from a plant, or a marine organism, or a fungus, or a strain of bacterium then the value of that organism is also markedly enhanced. Many alkaloid-containing plants are highly valued because their active constituents are beyond current commercial synthetic capabilities. For example, in many areas of the world, the periwinkle, *Catharanthus roseus* (L.) G. Don (Apocynaceae), is a weed, in other areas it may find value as a popular, decorative ornamental. Frequently, there is no realization

of the profound medicinal properties of its bisindole alkaloids as cancer chemotherapeutic agents, and thus its commercial value (Taylor and Farnsworth, 1973).

It is important to realize that while plant alkaloids comprise about 15.6% of the known natural products, they constitute almost 50% of the plant-derived natural products of pharmaceutical and biological significance. When the *in vitro* and *in vivo* biological data from the NAPRALERT^{sm} database are examined, this involvement in therapeutics is even more remarkable because a significant paucity of the biological evaluation of alkaloids is apparent. Of the 21 120 alkaloids from higher plants, 2291 have been evaluated in a single bioassay, and only 1995 have been evaluated in between two and five bioassays. Even more remarkably, only 167 alkaloids have been tested in 20 or more *in vitro* or *in vivo* bioassays. Thus, **over one-third (35.9%) of the alkaloids which have been examined biologically in 20 or more assays are pharmaceutically significant!** The vast potential for drug discovery from known alkaloids becomes apparent when one realizes that, in contrast, and based on published data, 16 132 alkaloids (76.4%) have yet to be examined in a single biological assay (Table 10).

Table 10. The biological evaluation of alkaloids from higher plants[a]

Number of biological tests	Number of alkaloids
0	16 132
1	2291
2–5	1995
6-10	366
11-15	119
16-20	50
20+	167
Total	21 120

[a] Data from the NAPRALERT Database, December 2000.

One critically significant question to answer for future drug discovery and development is whether there are likely to be new natural template molecules to be found for the world's major diseases? While the substantial level of effort devoted to plant anticancer agents over the past 30 years has demonstrated that this is undoubtedly the case (Cragg and Boyd, 1996; Cragg *et al.*, 1997b), for other major disease states it remains to be determined. Several years ago we initiated a small programme on the discovery of antimalarial agents from plants used for fevers in the Traditional Medicine system of Thailand. The goal was two-fold: (i) to seek new molecules for potential *in vivo* evaluation, and (ii) to demonstrate the significance of using current ethnomedical information for drug discovery purposes. Two plant families were excluded from evaluation, the Simaroubaceae (quassinoids) and the Meliaceae (limonoids), because of the high level of cytotoxicity of their known antimalarial metabolites (O'Neill *et al.*, 1988; Kigodi *et al.*, 1989; Phillipson and Wright, 1991). The results from the programme identified three alkaloid skeleta which were shown for the first time to possess selective antimalarial activity (Angerhofer *et al.*, 1992; Cordell *et al.*, 1994): the aporphines (Cordell *et al.*, 1994), the bisbenzylisoquinolines (Lin *et al.*, 1993; Likhitwitayawuid *et al.*, 1993a, 1993c; Angerhofer *et al.*, 1999), and the 5,10b-ethanophenanthridines (Likhitwitayawuid *et al.*, 1993b).

Multiple drug resistance (MDR) has become a very significant issue in pharmacotherapeutics and the journal *Drug Resistance Updates* regularly summarize these clinical developments. The number of disease states which are experiencing various levels of drug resistance is steadily increasing, and includes malaria, various forms of cancer, HIV and tuberculosis, as well as a number of fungal and bacterial infections (Henry, 2000). There are two classic compounds which are used in biological studies related to overcoming MDR, verapamil and the yohimbe alkaloid reserpine (Endicott and Ling, 1989). To date there have been few systematic studies designed to illuminate new natural products capable of overcoming MDR (Kim *et al.*, 1995). In our National Cooperative Natural Product Drug Discovery Grant programme we chose to include in our panel of biological assays of plant extracts an assay which had the potential for an extract to reverse the resistance of a genetically engineered human cancer (KB) cell line to vinblastine (Cordell *et al.*, 1991). The number of extracts capable of exerting this biological response was very small. However, four different alkaloid systems, the *Aglaia* bisamide alkaloid, piriferine (Saifah *et al.*, 1993), some iboga indole alkaloids from *Peschiera laeta* (Mart. ex A. DC.) Miers (Apocynaceae) (You *et al.*, 1994), the aporphine alkaloid roemerine (You *et al.* 1995), and the quinoline alkaloid, acronycine (You *et al.*, 2001), were found to exhibit this activity (Cordell *et al.*, 1998). More systematic studies in this area are clearly warranted as a response to enhanced drug resistance and the impact on the health care system and morbidity that will occur in the future.

So, is there another *Catharanthus, Atropa, Camptotheca, Rauvolfia, Cinchona, Papaver* or *Taxus* species waiting to be discovered and developed from somewhere on Earth? For the future of human health, the answer must be 'Yes'. Our pertinent questions then are: how many such plants are there, how will they be discovered, and when?

The Fit of Alkaloids in the Drug Discovery Process

The possibility of finding novel lead compounds has been greatly enhanced through the recent developments in combinatorial organic synthesis (Warr, 1997; Dolle and Nelson, 1999; Floyd *et al.*, 1999; Sun, 1999; Borman, 1999a, 2000) and high throughput screening (HTS) methods (Warr, 1997). As we have discussed, in the present climate of the pharmaceutical industry, the drug discovery process to elicit 'hits' is focused on strategies to evaluate the highest degree of chemical diversity possible in chemical libraries against the respective automated bioassays in order to maximize the coverage of the three-dimensional space of a receptor. Natural product compounds and extracts from plant and marine sources are rarely more than 3% of the total chemical library being biologically evaluated (Cordell, 2000). Frequently, groups of compounds from the total chemical library are clustered strategically so that screening can be made more effective than a totally random approach. Strategies for involving natural products in this process have been described (Schmid *et al.*, 1999; Grabley *et al.*, 2000). But, in order for natural products, either extracts

or compounds to be included, there must be a notion of what natural products offer structurally which differs from synthetic compounds (Henkel *et al.*, 1999), and what represents a 'drug-like' molecule in the situation of HTS. Several attempts have been made to develop concepts of what constitutes 'drug-likeness' in various subsets of compounds (Ajay *et al.*, 1998; Cummins *et al.*, 1996; Lipinski *et al.*, 1997). We will examine one of these and apply it to the pharmaceutically significant alkaloids.

What are some of the key characteristics of a typical drug? Based on an analysis of the World Drug Index of 50 427 compounds, Lipinski and co-workers (1997) pared this list to 2245 compounds which were considered to have superior physicochemical properties. Statistical evaluation of certain of the properties of these compounds suggested four properties which an orally available drug is very likely to possess. These factors include: (i) the molecule has fewer than 5 H-bond donors (expressed as the sum of OHs and NHs), (ii) there are fewer than 10 H-bond acceptors (expressed as the sum of Ns and Os), (iii) the molecule has a molecular weight of less than 500, and (iv) that log P (an absorption-permeabilty factor) (Moriguchi *et al.*, 1992) is less than 5. It was recognized that compounds that serve as substrates for biological transporters were exceptions to these rules. In addition to vitamins, three classes of drugs fell outside these parameters, antibiotics, antifungals and cardiac glycosides.

Are alkaloids 'drug-like' molecules? Can we speak of alkaloids in these terms? What are the chemical and physical characteristics of alkaloids which might make them a very special group of natural products on which to focus for future biological evaluation? First among these considerations is that compared with acetogenins, terpenoids and phenylpropanoids, alkaloids show more substantial skeletal structural and functional group diversity. Thus the 135 500 plant-derived natural products represent about 5750 total skeleta. Yet the alkaloids, which comprise 21 120 of the structures (15.6%), possess 32.5% (1872) of the total skeleta. This translates into greater pharmacophoric unit diversity and therefore a higher probability that a biological response will be displayed against a wider range of molecular target sites. The range of structures of alkaloids, from linear chains to planar, multi-ring systems, to globular molecules, runs the gamut of conformational rigidity and flexibility, allowing a vast range of possibilities for enzyme–substrate interaction. Most alkaloids have single or multiple chiral centres, and it is extremely rare that they occur in racemic mixtures. Rather, they are usually isolated with a very high degree of optical purity of one enantiomeric form. This also explains, in part, why alkaloids such as sparteine and quinine are receiving substantial attention as critical components in directing the enantiomeric stereoselectivity of synthetic reactions (Song, 2000).

It is pertinent to examine Table 8, the list of alkaloids of pharmaceutical and biological significance, with respect to the Lipinski Rules described above. Analysis shows that the average molecular weight of the 60 alkaloids listed is 348.9 Daltons, the number of NH and OH groups/alkaloid is 0.97, and the average number of N and O atoms/alkaloid is 5.55. Thus each of these parameters fall well within the conclusions for a molecule to be 'drug-like'. The main alkaloids which lie outside the parameters are aconitine, rescinnamine, reserpine, taxol, vinblastine and vincristine.

There are a number of other qualities of alkaloids which make them attractive for very detailed biological investigation. Alkaloids are of typically moderate molecular weight (250–600 Daltons), and thus are amenable to standard techniques of purification and spectral analysis to meet Federal drug standards (compare polysaccharides and proteinaceous materials). From what is known already, alkaloids display a wide range of biological responses, can have extremely high potency (nM range), and may serve as biological standards for enzymatic or receptor-based processes.

The basicity of most alkaloids means that they can readily be made substantially more water-soluble (and therefore probably more bioavailable) through salt formation. In addition, the substantial range of functional group diversity permits modifications which can introduce groups capable of modulating biological activity, of reducing lipophilicity or, if necessary, increasing lipophilicity. Finally, and for the long term benefit of the health of future generations, probably the most important aspect of their development is that alkaloids are derived from a sustainable resource.

The Issues and Opportunities for Alkaloids in Lead Discovery

Alkaloids, like other natural products, come with both opportunities and issues for consideration. We have already seen that alkaloids are not present in every plant, nor in every part of an alkaloid-containing plant. We have also seen that, from a structural perspective, alkaloids are distributed chemotaxonomically in a manner which reflects genetic capacity for synthesis at a particular point in time. Thus, the distribution of alkaloids when assessing plant materials for potential biological evaluation is certainly a matter for consideration. In addition, alkaloids are usually only present at a low level of an extract, possibly only 1%–2% of the weight of the dried plant material. Deconvolution (dereplication) of biologically active extracts for novel active compounds is a continuing issue in natural product drug discovery programmes (Corley and Durley, 1994). This concern is even more significant for extracts which contain alkaloids (because of the content level), and argues for the evaluation of concentrates of alkaloids from plants in chemical libraries for HTS. Strategies for deconvolution have therefore become an essential aspect of natural product drug discovery programmes from chemical (Aszalos and Frost, 1975) and biological (Beulter *et al.*, 1993) perspectives. Even more important now for natural product discovery programmes is to link the chemical and biological facets chromatographically in time, and then to use that information to compare with chemotaxonomic and biological databases (Cordell *et al.*, 1997; Cordell and Shin, 1999). We are also aware, after the taxol development problem (Suffness, 1995), that it is important to assure an adequate source and supply of active compound for the early chemical and biological stages of turning a 'hit' into a 'lead'. This factor alone is a critical consideration in the development process for natural products in advanced pharmacological and clinical studies.

Adequate chemical and biological evaluation of alkaloid-containing plants requires collection of the necessary plant material. Issues of negotiated access to the biome now dominate the phytochemical and the discovery aspects of natural product chemistry (Cragg *et al.*, 1997a). Unless these matters are resolved in a long-term equitable manner by all of the parties involved, the role of natural products generally in the process of drug discovery will be substantially diminished (Cordell, 1995b, 2000). That is the subject of a quite separate discussion.

On the other hand, there are a number of significant opportunities available for alkaloids in lead discovery. There is substantial ethnomedical and ecological information which remains unexplored from a biological perspective, and involves plants which might contain alkaloids as their active principles.

The active principle(s) of many of the phytotherapeutical (botanical supplement) and traditional medicine preparations which are currently available globally are unknown, yet may well be alkaloids. Using the same strategies being applied for the dereplication of biologically active extracts in discovery programmes, such as HPLC/ESMS linked to bioassay systems (Cordell and Shin, 1999), there is the opportunity to standardize, chemically and biologically, those preparations. This will undoubtedly open up new avenues for drug discovery and development, as it has in the past.

There is the structural diversity of alkaloids. As indicated previously, there are at least 1872 alkaloid skeleta known among the 21 120 alkaloids derived from higher plant sources (Table 3). This established molecular diversity is an extremely important aspect considering the number of alkaloid-containing plant species yet to be investigated chemically or biologically, and consequently the probable molecular diversity remaining to be disclosed. There are a significant number of plants which have received little attention for their alkaloids, even though they are in plant families which have been demonstrated to have substantial alkaloid content. For example, Table 11 shows those plant families with more than 20 alkaloids isolated where less than 10% of the genera have been studied for their molecular diversity. Similarly, Table 6 indicates, for the highest yielding, alkaloid-containing families by species, the approximate number of species in the family remaining to be examined.

As a result of the information developed on about 3 billion base pairs from the human genome project, it is estimated that well over 117 000 new gene targets for a variety of diseases will become available in the next few years as tools for the discovery of new molecular entities (Fannon, 1996). What this will mean in terms of drug discovery is the subject of much speculation, but the excitement is that clearly there are many, very different, fundamental philosophical approaches to using these target genes (Borman, 1999b; Eisenberg *et al.*, 2000; Pal, 2000). Some efforts, for example, may target one particular gene or possibly a cluster of genes which may be related to a particular disease state. Libraries of purified chemical entities and of natural product extracts will be used to monitor gene expression in microarrays (Lockhart and Winzeler, 2000). As noted earlier (Table 10), the structural diversity of alkaloids remains largely untapped from a biological perspective. Of the 21 120 known alkaloids, only 702 (3.3%) have been evaluated in more than five bioassays. Consequently, if diverse groups of alkaloids can be formatted in a manner appropriate for such screening, a significant opportunity for alkaloid-based drug discovery will be available.

New opportunities for traditional medicine may also occur through genomics. For example, given the philosophical underpinnings of multicomponent traditional Chinese medicines which have alkaloid-containing plants as its base, will it be possible to establish the multiple gene targets of such a prescription? Will this be a way to demonstrate that such prescriptions are capable of modulating more than the overt symptomatology, and thus enhance their validity and value? Will these discoveries lead to the characterization of standardized,

Table 11. Some poorly evaluated alkaloid-containing families by genus[a]

Family	Genera studied/total genera	% Studied	Number of alkaloids isolated
Orchidaceae	18/1000	1.8	53
Theaceae	1/40	2.5	54
Malpighiaceae	2/60	3.3	22
Arecaceae	7/200	3.5	21
Crassulaceae	2/25	4.0	47
Flacourtiaceae	4/85	4.7	21
Poaceae	36/500	7.2	256
Asclepiadaceae	19/250	7.6	180
Bignoniaceae	8/110	8.0	53
Malvaceae	6/75	8.0	48
Proteaceae	6/75	8.0	61
Verbenaceae	8/100	8.0	24
Asteraceae	92/1100	8.4	705
Acanthaceae	22/250	8.8	92
Gentianaceae	7/75	9.3	25
Araliaceae	7/70	10.0	55
Campanulaceae	7/70	10.0	53
Icacinaceae	5/50	10.0	26
Loranthaceae	7/70	10.0	20
Nyctaginaceae	3/30	10.0	22

[a] Families with 20 or more alkaloids isolated where less than 10% of the genera have been studied for their alkaloids.

multiple component alkaloid mixtures which may elicit responses from a diversity of disease-related genes, and thus be more substantially beneficial?

For alkaloids to fulfil their role in the immediate future technology of drug discovery, the range and complexity of alkaloid structural diversity must be enhanced. There are at least four approaches to achieving this goal: (i) enhanced targeted chemotaxonomic and phytochemical exploration, (ii) combinatorial synthesis, (iii) combinatorial biosynthesis, and (iv) chemical diversification. Some brief comments on each of these approaches are warranted.

We have seen that current drug discovery strategies relate to the optimization (not necessarily maximization) of chemical diversity for biological assessment. Thus any effort to expand the chemical knowledge of alkaloids is likely to have a significant impact on their biological potential from a discovery perspective. From the aspect of higher plants, there are three groups of plant families: those which have structurally characterized alkaloids, those which contain uncharacterized alkaloids, and those which have not yet been investigated for their alkaloid potential. These groups of families merit selective attention in a manner which can potentiate the link between chemistry and biology. In order to optimize the conformational diversity at the biological interface, those plant families which produce a high diversity of alkaloid skeleta are of special interest. Table 8 summarizes those plant families which produce more than 10 different classes of alkaloid skeleta, together with the number of individual alkaloids of each structure class that have been isolated to date.

Plants which have demonstrated the potential to afford alkaloids are continuing to afford new alkaloids and new alkaloid skeleta. Plant families which contain uncharacterized alkaloids must be examined chemically for the potential to yield new structural classes which could present new pharmacophoric moieties. Finally, representative genera of the 153 plant families which have never been evaluated for alkaloids must be collected while they are still available and examined for their potential to yield new alkaloids.

The powerful technique of combinatorial synthesis has been used very sparingly on natural products thus far (Nicolau *et al.*, 2000). In the case of alkaloids, there is one study which reported on the combinatorial synthesis of yohimbine and rauwolscine derivatives (Atuegbu *et al.*, 1996), one which produced a very limited number of taxol (paclitaxel) derivatives (Bhat *et al.*, 1998), and another which yielded 400 taxoid derivatives (Xiao *et al.*, 1997). Several of the major alkaloid classes would appear to be prime candidates for such studies, including the tropane (e.g. 6β-hydroxy-nortropan-3β-ol), aconitine, pilocarpine, physostigmine, veratramine, pyrrolizidine, quinolizidine and pinidine systems.

Various aspects of biotechnology have had, and will continue to have, a dramatic impact on alkaloids and their availability as drugs (Walton, 1992; Kutchan, 1998; Verpoorte *et al.*, 1998). For example, introducing an ornithine decarboxylase gene from yeast into transformed root cultures of *Nicotiana* spp. using *Agrobacterium rhizogenes* increased nicotine production (Hamill *et al.*, 1990). Engineered hairy root cultures of *Atropa belladonna* L. (with encoded hyoscyamine 6β-hydroxylase) permitted higher yields to be obtained of the more valuable alkaloid scopolamine (Hashimoto *et al.*, 1993). Some of these rapid advances in the molecular genetics of alkaloid biosynthesis were reviewed by Kutchan (1998).

Enzymes from alkaloid biosynthesis have also been cloned and expressed in yeast and in insect cell cultures (Dittrich and Kutchan, 1991; Pauli and Kutchan, 1998; Lister *et al.*, 2001) and were shown to have the same activity as the native enzyme. Such studies allow for explorations of the substrate specificity of the enzymes in a way which was heretofore impossible (Kutchan and Dittrich, 1995). An extension of this scenario is into the synthetic transformation area, and the use of a renewable cell system for important chemical steps in a synthetic pathway.

Combinatorial biosynthesis, a process in which the genetic information concerning a pathway is altered, has attracted substantial interest in recent years, particularly in relation to the polyketide pathway in *Streptomyces* (Borman, 1998b). As more information accrues regarding the genes of alkaloid biosynthesis, one can anticipate the shift of the production of critical alkaloids to other faster growing, more controllable organisms, as has been achieved for strictosidine synthase (Kutchan, 1998). The strategies and techniques which yielded new erythromycin derivatives (Donadio *et al.*, 1991) have evolved to explore the formation of novel polyketides from *Streptomyces* polyketide synthase (Yu *et al.*, 1998) and chalcone synthase (Abe *et al.*, 2000), and have yielded a β-amyrin synthase from a lupeol synthase (Kushiro *et al.*, 2000). These techniques will undoubtedly be applied to form new alkaloids through the resequencing of modular steps in a particular biosynthetic pathway. It will soon be possible to shift the gene sequences and modulate substrate specificity to produce new alkaloid skeleta from unnatural substrates which can specifically target controlled modifications of pharmacophoric moieties. A recent study of four recombinant *O*-methyl transferases for their substrate specificity is therefore of significant interest (Frick *et al.*, 2001). Polyfunctional, enzymatically controlled drug design of molecules based on genomic targets, which also incorporate structural elements to address drug resistance, might become a feasible strategic synthetic target. The future of synthetic organic chemistry is clearly in modifying the enzymes of natural product biosynthesis for use as renewable agents for otherwise unachievable processes; the berberine bridge enzyme system is an example (Steffens *et al.*, 1985).

The enzymatic studies of alkaloid biosynthesis have yielded some useful results for the biotransformation of alkaloids (Lister *et al.*, 2001). These biotransformations may have important implications for the production of compounds of pharmaceutical significance from more readily available precursors when synthetic conversions are low yielding (Lister *et al.*, 1999). When such enzymes have broader substrate specificity, synthetic transformations may also be achieved with systems that were originally discovered based on non-alkaloidal pathways. One example is the use of (3–17)β-hydroxysteroid dehydrogenase and morphinone reductase, expressed in *E. coli*, to produce hydromorphine from morphine (Lister *et al.*, 2001). One should expect that such systems will continue to be expanded, in terms of chemical diversity, host organism, and the number of constitutive steps. Degradative biotransformation of nicotine has also yielded substituted pyridines of use in the synthesis of difficult to prepare pyridinyl derivatives for biological evaluation (Roduit *et al.*, 1997).

For now though, one of the easiest and fastest ways to enhance natural product structural diversity, including that of alkaloids, is to conduct selected, simple chemical reactions, such as oxidations, reductions, alkylations, hydroxylations and esterifications, on plant (or marine) extracts (Cordell, 2001). No publications have appeared in this area as yet, although the potential is high to produce a wealth of new entities in a simple manner, under mild conditions, in high yield, for biological evaluation.

Finally, it is well established that oil is a finite resource. While the main use of chemicals from fossil hydrocarbons is for plastics, textiles, polystyrene, dyestuffs and nylon, synthetic drugs are also derived from this source; a source which may not last for more than the next 65 years based on anticipated reserves and their daily use (Evans, 1999). Dwindling supplies will undoubtedly have a profound effect on the processes used for the production of drugs, and consequently their cost. As other industries are presently examining plant materials for alternative, sustainable sources of oils, so the pharmaceutical industry will be required to examine plants as a sustainable source for chemicals from which medicinal and biological agents can be derived (Cordell, 2000). New strategies for the process of discovering sustainable drugs will need to be developed, and the inclusion of both traditional medicines and alkaloid-containing plants in that process will be critical.

Conclusions

The need to consider how drug discovery can focus on a more sustainable drug supply has been described based on an important group of natural products—the alkaloids. Alkaloids are presently at the cornerstone of global health care, either as individual agents, as semisynthetic derivatives, as pharmacophoric units in numerous synthetic drugs, or as active principles in various traditional medicines. Alkaloids are also fundamental to some of the world's most significant socio-economic issues, including euphoric stimulant drug and tobacco addiction.

Two aspects of the potential for alkaloids to provide pharmaceutical and biological agents in the future have been highlighted: that the vast majority of the known alkaloids have been very poorly evaluated biologically, and that there are many plants containing alkaloids which remain uninvestigated chemically and biologically. In addition, the superior potential of alkaloid-containing plants and their contained alkaloids to continue to contribute to the health and welfare of future generations in a sustainable manner has been demonstrated in this article based on the drug-likeness of alkaloids, their skeletal diversity, and their dominance in current pharmaceutical agents. However, in order to explore fully the economically useful potential for the alkaloids, innovative strategies, taking full advantage of the extant chemical, biological, technological and biotechnological developments, will be necessary.

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