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Structure elucidation of organic compounds from natural sources using 1D and 2D NMR techniques

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Abstract

In our continuing studies on Lamiaceae family plants including *Salvia*, *Teucrium*, *Ajuga*, *Sideritis*, *Nepeta* and *Lavandula* growing in Anatolia, many terpenoids, consisting of over 50 distinct triterpenoids and steroids, and over 200 diterpenoids, several sesterterpenoids and sesquiterpenoids along with many flavonoids and other phenolic compounds have been isolated. For *Salvia* species abietanes, for *Teucrium* and *Ajuga* species neo-clerodanes for *Sideritis* species *ent*-kaurane diterpenes are characteristic while nepetalactones are specific for *Nepeta* species. In this review article, only some interesting and different type of skeleton having constituents, namely rearranged, nor- or rare diterpenes, isolated from these species will be presented. For structure elucidation of these natural diterpenoids intensive one- and two-dimensional NMR techniques (^{1}H, ^{13}C, APT, DEPT, NOE/NOESY, ^{1}H–^{1}H COSY, HETCOR, COLOC, HMQC/HSQC, HMBC, SINEPT) were used besides mass and some other spectroscopic methods.

Keywords: Labiatae family plants; Terpenoids; Diterpenoids; Structure elucidation; NMR and other spectroscopic techniques

1. Introduction

Terrestrial and marine plants have served humankind as natural sources of medicinal agents since ancient times. Today, natural products and their derivatives represent about 50% of all drugs in clinical use, higher plant-derived natural products represents half of this percentage [1]. In the last decades, developments in spectroscopic techniques at molecular levels, improvements in immunology and enzymologie as well as in investigating structure–activity relationships (SAR studies) and sensitive bioassays led to discover many important drugs originated from natural sources. As the most important source of new drugs, plants presented several recent drugs which are derived from their secondary metabolites or prepared from them as semi-synthetic and synthetic derivatives for clinical uses. Plant-derived organic compounds include important anti-cancer and antimicrobial agents, vinblastin, vincristin and their new semi-synthetic derivatives, such as vindesine, vinorelbine [2], and campthotecin (topotecan and irinotecane), taxol as anti-cancer, quinine and artemisinin as anti-malarial agents are some of well known examples [1–4]. In structure elucidation of organic compounds, developments in NMR techniques during the last two decades were not denied which allowed to discover many new natural organic compounds. Almost all molecular spectroscopic techniques have been used by natural product chemists, but without NMR, no new structure can be dissolved.

Turkey has a very rich and diverse flora which consists of ca. 10,000 plants, represented by 173 families and 1225 genera [5,6]. One of the most endemic (33%) families is Lamiaceae (Labiatae), represented by about 250 genera and 3000 species in the world. In Turkey, Lamiaceae family is represented by 45 genera and 558 species, 28 genera are

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widely distributed and 247 species are endemic (growing only in a specific location) [5,6].

Our studies on Lamiaceae family plants have been continuing over 30 years, and structure elucidation studies of their secondary metabolites which consist of namely terpenes (mono-, sesqui-, di,- and triterpenes) and flavonoids and other phenolics have been carried out using intensive 1D and 2D NMR techniques along with other spectroscopic (IR, UV, mass and if available single crystal X-ray) techniques. Particularly, terpenoids are the largest and most widespread class of secondary metabolites. In general, terpenes are the compounds containing an integral number of C_5 units and all terpenoids can be considered to be derived from C_5 unit isoprene (2-methyl-1,3-butadiene), therefore terpenoids are classified into mono-, sesqui-, di-, sester-, tri-, and tetraterpenoids having 2, 3, 4, 5, 6 and 8 isoprenoid C_5 residues, respectively. In Lamiaceae family plants, terpenoids, especially diterpenoids were found to be most dominant secondary metabolites and isolated especially from the dichloromethane and/or acetone extracts studied by our group.

2. Experimental

2.1. Plant material

All Lamiaceae family plants, investigated by us, collected from Turkey in their flowering season (May–August). Their voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, Istanbul University except *Sideritis* species which were either in Anadolu University, Herbarium of Faculty of Pharmacy or in special collection of Tuncay Dirmenci from Faculty of Science and Arts at Balıkesir University, or other Herbariums, mentioned in the cited references.

2.2. General

The IR spectra were taken in $CHCl_3$ on Perkin Elmer 983 spectrophotometer, in general. The NMR (^1H, ^{13}C BB, APT, DEPT, HETCOR, COLOC, HMQC, HMBC) spectra were recorded on either a Bruker 200 MHz or a Varian 400 MHz NMR spectrometer, and mostly in $CDCl_3$. In some cases, especially some 2D NMR experiments were carried out by using different NMR spectrometers in Turkey or abroad shown in the related literatures through the text. TMS was used as an internal standard and chemical shifts were given as δ (ppm), and the coupling constants (J) were reported as Hz. The mass measurements, particularly HRMS were measured on a VG-ZabSpec mass spectrometer at Marmara Research Center at TUBİTAK, Gebze. Column chromatography (CC) was carried out on Silica gel (Kieselgel 60, 0063–0.200 mm, Art. 7734, Merck) filled columns and for preparative TLC, Kieselgel 60 F_{254} (0.5 mm thickness, Art. 5554, Merck) plates were used, and visualized with UV light and sprayed with ceric sulphate reagent, and heated.

2.3. Extraction, isolation and purification

The air dried and powdered plants (either whole plant or aerial parts/roots) were extracted with different solvents mostly with dichloromethane or acetone, depending on the plant genus. In some cases, extraction was carried out by treating the plant first with a non-polar solvent (such as hexane), followed by dichloromethane or acetone, then methanol exhaustion. For each plant, the extraction and isolation procedure was reported in the related publications.

3. Results and discussion

3.1. Isolation and purification of diterpenoids

Plant extracts are prepared by extraction of either whole plant or aerial parts and roots separately with a proper solvent, mostly acetone or dichloromethane. In the previous years, we also used Soxhlet extraction, however, possibility of the presence of sesquiterpenes and some easily destroyed diterpenes, maceration at room temperature is more accepted extraction method. For the rough separation of terpenoids, especially diterpenes and triterpenes Silica-gel filled columns are used. Elution of the columns are started with hexane, and followed by a gradient of dichloromethane or acetone arising 100%, then the amount of methanol increased slowly up to 10–20%, rarely 100%. The TLC spots are detected with UV light and sprayed with seric sulphate reagent [cerium (IV) sulphate in aqueous methanol], and heated. Similar fractions were combined and purified on preparative Silica-gel TLC plates or silica based HPLC columns and/or Sephadex LH 20 column, or flash chromatography or chromatotron, and visualized by serium sulphate reagents. Some Lamiaceae plants have been investigated by our group shown below:

Species	Number in Turkey	Investigated species	Isolated compounds	Isolated diterpenoids
Salvia	(86)	51	288	183
Sideritis	(46)	12	45	35
Teucrium	(27)	4	32	15
Ajuga	(11)	3	22	6
Nepeta	(33)	2	12	–
Lavandula	(2)	2	14	2

Among Lamiaceae family plants, the most studied genus is *Salvia* by our group. There are over 900 *Salvia* species in the world, while this genus is represented by 90 species in Turkey, half of them being endemic. They are important medicinal plants due to their diverse secondary metabolites [7–10], especially abietane diterpenoids. *Salvia* (sage) species have been used since ancient times with more than 60 different ailments ranging from aches to epilepsy, mainly to treat cold, bronchitis, tuberculosis, hemorrhages and menstrual disorders. Sage is also used to preserve foods, especially meets and cheeses due to antioxidant properties.

3.1.1. Salvia diterpenoids

Abietane diterpenes are the main constituents of Anatolian *Salvia* species while neo-clerodanes were found commonly in American *Salvia* species [7]. Since 1970s we have been working on *Salvia* species, and studied more than half of them (51 species) [8–10]. In Turkish *Salvia* species, most abundant abietanes have a phenolic C-ring, such as ferruginol (Fig. 1) and derivatives. In the ^{1}H NMR spectra of these common abietanes, five methyl groups are observed, three of them as singlets while the other two appeared as doublets together with a methine proton as a septet which belonging to an isopropyl side chain attached to ring C, in general. In some cases one methyl group, rarely two can be either are oxidized to acid or aldehyde or reduced to alcohol group or converted into a lactone moiety [11]. Royleanone, horminone, acetylhorminone and their analogues are also common abietanes with a *p*-quinone C ring moiety [12] in *Salvia* species. There are also *o*-quinone C ring containing abietanes, but not as common as *p*-quinone ones in *Salvia* species growing in Turkey [13]. Diterpenes were abundant in the roots of *Salvia* species while triterpenes and flavonoids are in the aerial parts. In fact, in *Salvia* genus, the diversity of abietane diterpenes is very rich, however, the diversity of triterpenes [8,10] or sesqui- [8,14] and sesterterpenes [15] is not.

One of the lactonized abietane diterpene was isolated from *Salvia wiedemanni* [11] which has a cross conjugated dienone ring system and a lactone ring. For structure elucidation of this compound, a series NMR experiments including COSY, DQ-filtered COSY, NOESY, DEPT, HETCOR and FLOCK and selective INEPT experiments were carried out besides CD, UV, IR and mass spectral measurements. The appearance of 20 carbons in the ^{13}C NMR by DEPT experiments (Fig. 2) and isopropyl group protons besides two additional methyl signals remained us the presence of abietane ring, but with a lack of one methyl group. A molecular ion at *m/z* 314.1854 requiring a molecular formula of $C_{20}H_{26}O_3$ with eight units unsaturation, suggested an abietane diterpene with two trisubstituted double bonds. The COSY and HETCOR spectra enabled the two isolated spin systems of C-1 through C-3 as three sequential methylene groups, and C-5 through C-7 consisting of a methine and two methylenes in sequence. Long range heteronuclear coupling was observed in the FLOCK spectrum between the low-field proton at δ 6.70, at the β-position of an α,β-unsaturated ketone, with the ketone carbonyl carbone at 184.4 ppm as well as the isopropyl methine carbone (δ 26.5) required that the isopropyl group be adjacent to this proton, with the ketone carbonyl also three bonds away. Further, an NOE was observed between this low-field olefinic proton and H-7 β in a difference experiment, supporting the location of this olefinic proton at C-14. Long range observations indicated the typical abietane nature of the A and B rings, but with the angular C-20 oxidized to a lactone carbonyl (δ 175.5), similar to that found carnosol or derivatives, with the remaining units of unsaturation (two double bonds and the ketone) located in the C ring. The location of the lactone carbonyl at C-20 was supported by long-range heteronuclear couplings to this carbonyl carbon (δ 175.5) from H-1α and H-5 observed in the FLOCK spectrum. An NOE between the higher field proton at δ 5.92 (H-11) and H-1α (δ 1.57) supported this structure. No coupling was observed between the two olefinic protons even in a long range COSY experiment which suggested their 1,4-arrangement on the ring [11].

By the observation of long-range heteronuclear couplings between H-11 (δ 5.92) with the oxygenated quater-

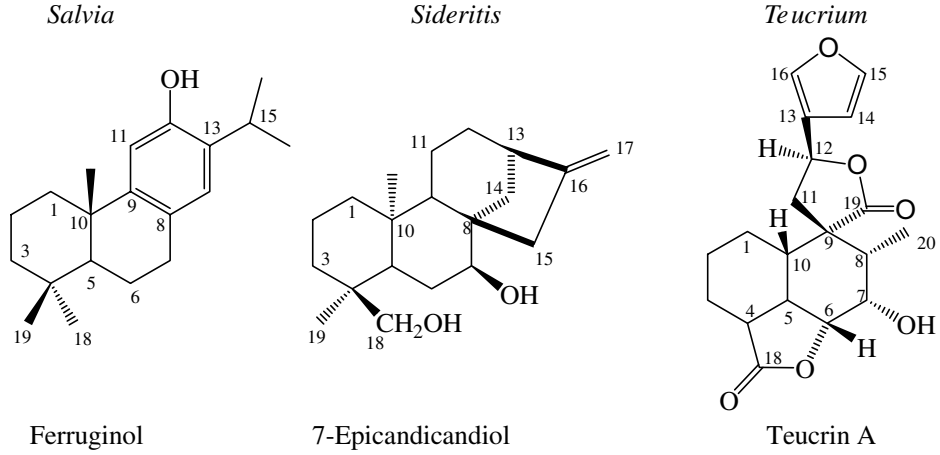


Fig. 1. Representative diterpenes for *Salvia* (abietane), *Sideritis* (ent-kaurane) and *Teucrium* (neo-clerodane) species.

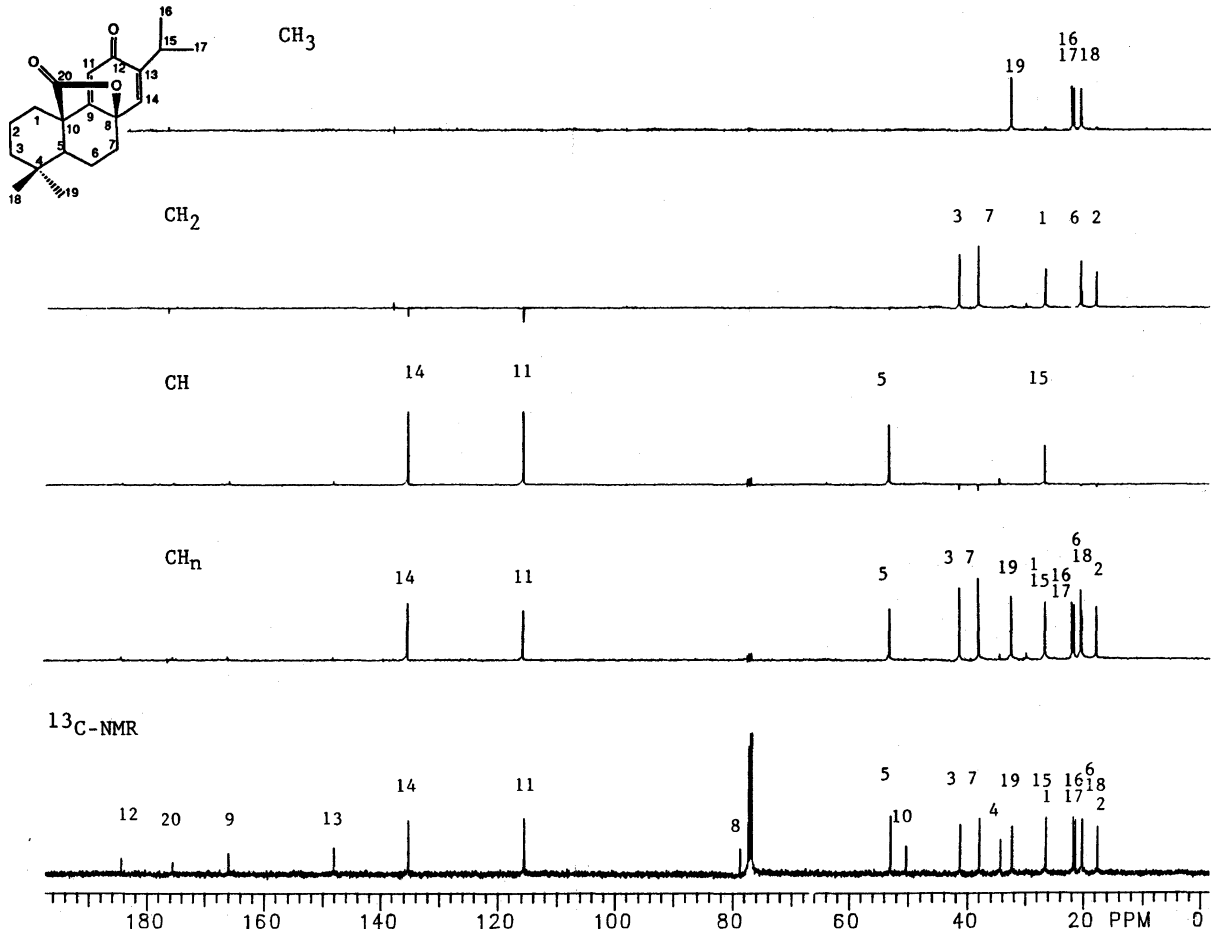


Fig. 2. ^{13}C NMR of wiedelactone (DEPT and BB decoupled experiments).

nary carbon at δ 78.7 (C-8) and δ 50.3 (C-10) as well as coupling between H-14 and C-9 confirmed the structure. Zinc reduction of the compound in acetic acid produced [11] the known diterpene pisiferic acid, confirming the absolute stereochemistry assignment.

The most interesting abietane diterpenes in *Salvia* species were rearranged abietanes and norabietanes, and NMR spectroscopy is very important tool to elucidate their structures. Among isolated over 170 abietane diterpenoids from Anatolian *Salvia* species, about 35 of them exhibited rearranged structure. Unfortunately, since we could not get them in proper crystal forms, their X-ray analysis cannot be realized yet. Aethiopinone and salvipisone were isolated from several Turkish *Salvia* species as known rearranged abietane diterpenes which have been first isolated from *S. aethiopis* growing in Spain [16,17].

Their analogues have been isolated from some *Salvia* species, especially from *S. candidissima* and *S. limbata.* Their oxo derivatives 1-oxoaethiopinone, 1-oxosalvipisone and 3-oxosalvipisone isolated from two subspecies of *S. candidissima* [18,19] while from *S. limbata*, as analogous of rearranged diterpenes aethiopinone or salvipisone, 12-hydroxysapriparaquinone, 3,12-dihydoxysapriparaquinone-1-ene, 2-hydroxysaprorthoquinone and limbinal (12-hydroxy sapriparaquinone-16-al) were isolated besides four new rearranged diterpenes limbinol, acetyllimbinol, salvilimbinol and 4-dehydrosalvilimbinol (Fig. 3) [20,21]. However, the last two compounds with irregular structures require more spectral measurements, since 2D NMR studies were not enough satisfactory in the meantime.

In addition, 6-dehydroyosgadensonol, 6-dehydro-13-*epi*-yosgadensonol as norsesterterpenes have also been isolated from the same plant with similar spectral properties to the previously isolated dinorsesterterpenes yosgadensonol and its 13-epimer (Fig. 4), isolated from *Salvia yosgadensis* [15,22]. Three new norditerpenes 6α-hydroxyambreniolide, 6α-hydroxynorambreniolide and 6α-hydroxy-8α-acetoxy-13,14,15,16-tetranorlabdane-12-oic acid were also isolated from *S. yosgadensis* extract (Fig. 4) besides known diterpenes ambreniolide and norambreinolide [15].

In fact, aethiopinone and salvipisone or their derivatives formed by the cleavage of the bond between C-4 and C-5 in abietanes, therefore their A ring became opened and they are named 4,5-seco-5,10-friedo-abietanes [23]. A few more step rearrangements on aethiopinone or derivatives led a new series rearranged abietanes, which have 7 or 8 membered ring A, isolated from Anatolian *Salvia* species by our group (Fig. 5). The first member of this series rear-

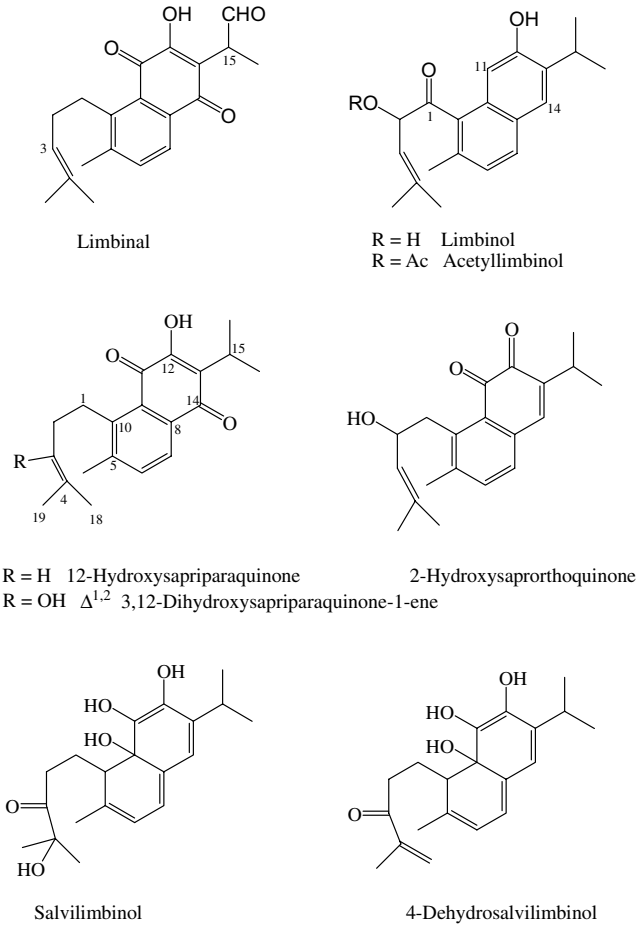


Fig. 3. Rearranged abietane diterpenoids isolated from *Salvia limbata*.

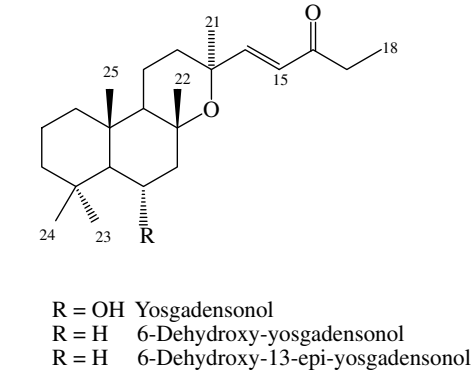


Fig. 4. Sesterterpenes isolated from *Salvia yosgadensis*.

ranged abietanes was microstegiol [24], isolated from the aerial parts of *S. microstegia*. Its structure was elucidated based on intensive NMR techniques (COSY, NOESY, APT, HETCOR, and SINEPT) (Table 1). In the ^{1}H NMR spectrum (in $CDCl_3$), the presence of an isopropyl group was observed with two doublet signals at δ 1.14 ($J = 7.0$ Hz) and 1.18 ($J = 7.0$ Hz) and a doublet of septet at δ 2.99 ($J = 6.9$ and 1.1 Hz). There was also an aromatic methyl singlet at δ 2.30 and a gem dimethyl at δ 0.76 (H-18) and δ 0.77 (H-19) as singlets, and three aromatic protons at δ 7.04 (d, $J = 7.7$ Hz), 6.94 (d, $J = 1.0$ Hz) and δ 6.87 (d, $J = 7.6$ Hz) corresponding to H-6, H-14 and H-7, respectively, suggesting the presence of an abietane type diterpene skeleton. However, the unusual scalar couplings between H-6 and H-20, as well as between H-7 and H-20 in the homonuclear COSY spectrum, clearly indicated a rearrangement of the abietane skeleton. Examination of the homonuclear COSY spectrum revealed the presence of three pairs of non-equivalent methylene proton signals at δ 3.58 and 2.76; 2.34 and 1.25; 1.78 and 1.40. The doublet of doublet of doublets at δ 3.58 (ddd, $J = 13.1$, 13.1, 2.2 Hz) which showed scalar couplings with three proton resonances at δ 2.76 (ddd, $J = 14.1$, 6.3, 2.2 Hz, H-1α and 1.78 (m, H-2α and 1.40 (m, H-2β was assigned to H-1β. From HETCOR experiments correlation between a carbon signal at δ 26.86 and the one methylene pair signals at δ 3.58 and 2.76 clearly indicated that this carbon signal belong to C-1 atom. Thus the appearance of benzylic carbon at 26.86 indicated no any substituent attached to this carbon, however, the highly deshielded benzylic proton at δ 3.58 belonged to this carbon (H-1β) existed the most distinctive property of this type of rearranged structures which followed by in the next examples of microstegiol analogous, such as candidissiol and salvibretol [25,26]. A coupling observed between H-3α (δ 2.34) and one of the gem-dimethyl groups at δ 0.76 led the assignment of these two methyl groups should be at C-4. But, based on COSY and NOE experiments results it was not possible to elucidate the connection between ring A and B/C rings. Therefore, construction of the carbon framework was made possible through the interpretation of a series of selective INEPT experiments. Irradiation of H-1α and H-1β, separately, consistently enhanced three carbon resonances at 143.29 (C-10), 139.40 (C-9) and 137.38 (C-5) exhibiting the linkage between C-1 and C-10. When H-2 protons irradiated separately, enhancement of the C-1, C-3 and C-4

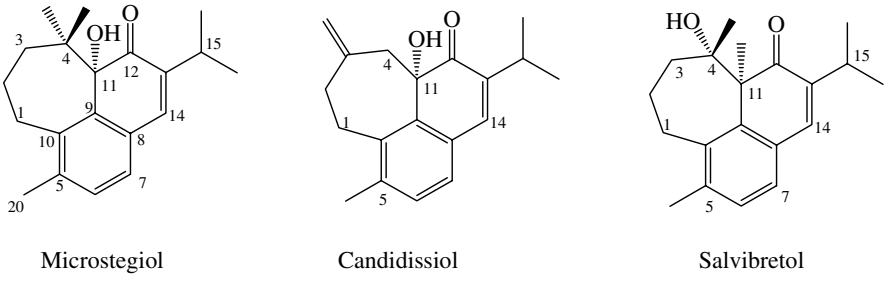


Fig. 5. Rearranged abietane diterpenoids isolated from some Anatolian *Salvia* species.

Table 1
^{13}C NMR spectral data of some rearranged abietane diterpenoids isolated Anatolian *Salvias* species

C	Microstegiol	Candidissiol	Salvibretol	1-Oxo-salvibretol
1	26.9	25.8	26.5	210.8
2	23.5	30.0	23.0	41.2
3	42.9	32.3	33.3	35.1
4	39.1	152.3	85.0	84.3
5	137.4	137.9	132.7	132.2
6	130.1	130.2	126.7	126.3
7	126.7	127.9	125.8	128.1
8	129.1	125.8	134.1	126.8
9	139.4	139.1	129.7	130.4
10	143.2	144.1	147.3	147.4
11	84.4	80.1	41.6	41.2
12	206.1	209.2	202.9	202.3
13	141.0	142.4	134.9	136.6
14	140.9	140.7	120.7	121.1
15	27.1	26.8	27.6	27.8
16	22.7	21.7	22.3	22.3
17	21.1	20.2	22.4	22.3
18	22.1	54.3	26.3	25.9
19	28.0	117.7	27.4	26.2
20	21.4	20.0	19.1	18.7

resonances was observed in addition to C-10 resonance. Respective irradiation of H-3α and H-3β enhanced the C-4 and C-11 resonances revealing the most important link between rings A and C. The observation of a NOE difference spectrum and the scalar coupling between H-1β and H-20 in the COSY spectrum substantiated that the carbon framework of the compound consists of a seven-membered ring A. Finally, the new compound, named microstegiol, might be existed from the abietane diterpene first by ring cleavage between C-4 and C-5 to afford aethiopinone-like abietane diterpene, followed by recyclization of C-4 to C-11 [24].

As the second rearranged abietane of this series, candidissiol was isolated from S. *candidissima* ssp. *candidissima* [25] for the first time which may arised by the possible rearrangement of aethiopinone (Fig. 6). In fact, its structure was fairly similar to microstegiol (Table 1), the main difference is the presence of an exocyclic methylene group, thus a new rearrangement of ring A led to formation of an eight membered ring. Both of subspecies of *S. candidissima* (ssp. *candidissima* and ssp. occidentalis) were found to be rich in rearranged abietanes [18,19,25].

In a later study, salvibretol was obtained from *Salvia montbretii* [26]. Its formation is considered through Wagner-Meerwein rearrangement from microstegiol. The ^{13}C NMR spectrum (Table 1) exhibited a diterpene structure with 20 carbon atoms consisting of five methyl, three methylene, four methine and eight quarternary carbon atoms. A diagnostic signal appeared at δ 3.83 (1H, ddd, $J = 15.0$, 13.0, 5.0 Hz), being typical for H-1β proton of a rearranged abietane diterpenes which was seen with the same multiplicity and similar chemical shift value to those of microstegiol and candidissiol. It was namely differed than microstegiol with one of methyl singlet signal which resonated at δ 1.62. Therefore, interchange between C-4 methyl and C-11 hydroxy group is clear, the possible mechanism for this, is shown in the Fig. 7.

In structure elucidation of this series compounds SINEPT experiments (Fig. 8) were very informative to determine three-bond away heteronuclear connectivities as well as two-bonds away ones. Irradiation of H-15 (3.35) enhanced C-14 (δ 120.7), C-16 (δ 22.3) and C-12 (δ 202.9). Irradiation of Me-18 (δ 1.15) enhanced C-4 (δ 85.0) and C-19 (δ 27.4), while irradiation of Me-19 (δ 1.62) enhanced C-4 (δ 85.0) and C-18 (δ 26.3); and all other enhancements verified the structure of salvibretol.

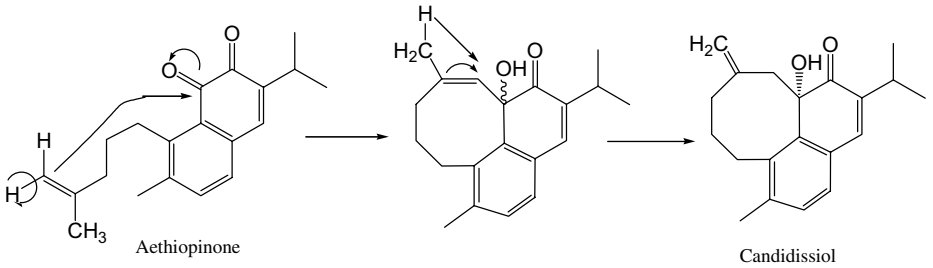


Fig. 6. Possible formation of candidissiol through aethiopinone.

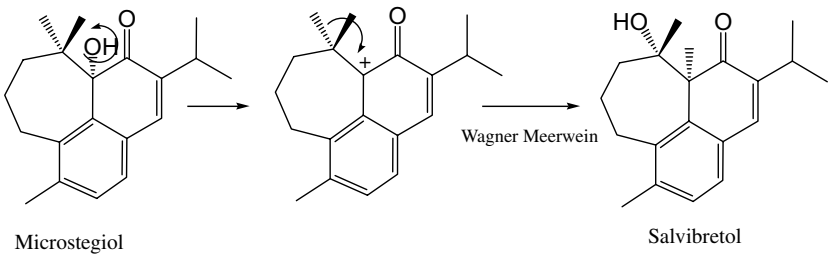


Fig. 7. Formation of salvibretol through Wagner-Meerwein rearrangement from microstegiol.

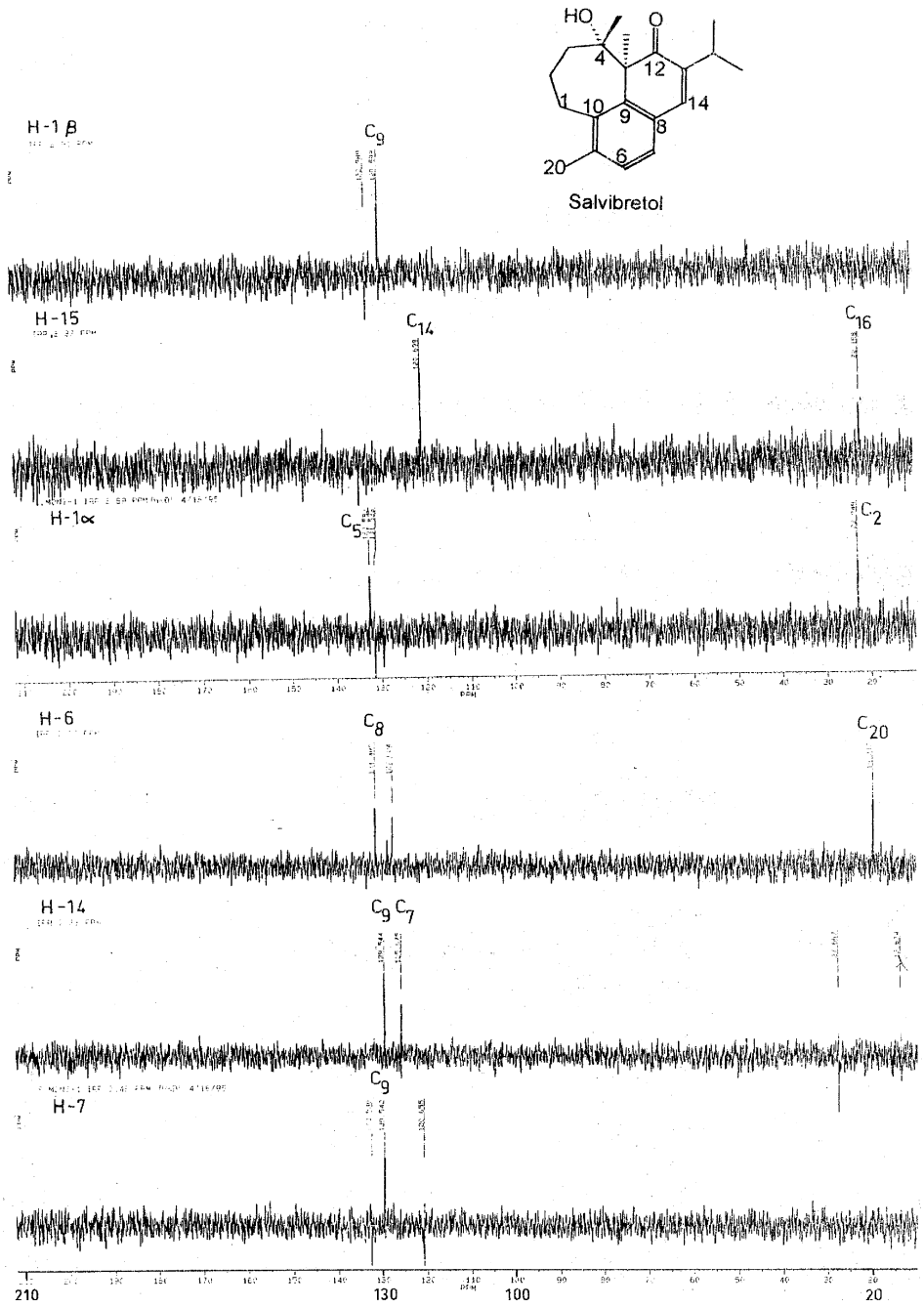


Fig. 8. Selective INEPT experiments of salvibretol (Irradiated protons are shown in the left-top side of each experiment, and in the irradiations, *J* value is chosen as either 6 or 8 Hz.).

From the same plant extract besides salvibretol, 1-oxo-salvibretol, microstegiol, candidissiol, 6-hydoxysalvinolone and 7-hydroxytaxodione and two dimeric abietanes 7,7–bistaxodione and 11,11-didehydroxy-7,7-dihydroxytaxodione have also been isolated (Fig. 9) [26].

The HREIMS of 7,7′-bistaxodione and 11,11′-didehydroxy-7,7′-dihydroxytaxodione gave a molecular ion peak at *m/z* 626.3720 and 626.3722, respectively, corresponding to molecular formula $C_{40}H_{50}O_6$. The presence of a fragment ion peak at *m/z* 313, which was assigned to half-of-the-molecule, indicated the dimeric structure of each compound. The other spectral data, particularly UV absorptions at 460, 360 and 318 nm for 7,7′-bistaxodione and 460, 380 and 320 nm for 11,11′-didehydroxy-7,7′-dihydroxytaxodione and NMR spectral data verified their symmetrical dimer nature [26].

In fact, icetexane diterpenes have been isolated from *Salvia* species very unusually [27], however, a natural noricetexane diterpene has been isolated from *Salvia multicaulis* [28] for the first time, named salvimultine (Fig. 10), and its structure was established as 1(10)-seco-2(10)-cyclo-icetexane by using 1D and 2D NMR

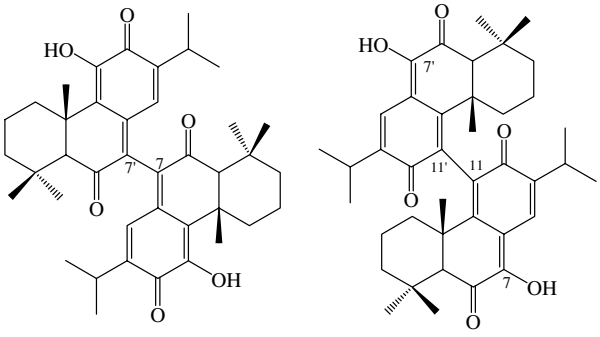


Fig. 9. Dimeric abietane diterpenoids isolated from *Salvia montbretii*.

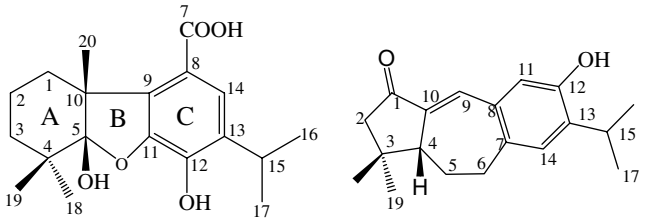


Fig. 10. Unusual rearranged diterpenes from *Salvia* species.

spectroscopy, including COSY, HETCOR, COLOC, and NOESY experiments besides HRMS. The other unusual diterpene salvicanaric acid has been first isolated from *S. canariensis* by Gonzalez et al. [29] as well as a few species among our investigated 51 Anatolian *Salvia* species (Fig. 10) [30].

The well known *Salvia* species, Chinese sage *S. milthiorhizza* afforded many nor-abietanes, called tanshinones. We have also isolated more or less similar type of norabietanes which contain 19 C atoms in their main skeleton (Table 2), instead 20 C [30,31]. This type of norabietane diterpenes are characterized with fully aromatized tricyclic rings (A, B, C) and two aromatic methyl signals of ring A, resonated between 2.3 and 2.6 ppm, and the lack of Me-20. The most important properties of our isolated norabietanes is to show antituberculous effect against *Mycobacterium tuberculosis*, which was higher than commercial antibiotics kanamycin, ethambutol, PAS and isonicotinic acid hydrazid showed. Among isolated four norabietanes (Fig. 11) from the acetone extract of *S. multicaulis* roots, particularly 12-demethylmulticauline showed high activity with a MIC (minimum inhibition concentration) value of 0.46 μg/mL. It exhibited a molecular ion peak at *m/z* 264.1522 corresponded to the molecular formula $C_{19}H_{20}O$, indicating 10° of unsaturation. The ^{13}C NMR (APT) spectrum (Table 2) correlated with the aromatic norditerpene structure giving signals for four methyl, seven methine and eight quaternary C signals. Among the methine signals, only one is aliphatic which belonged to isopropyl methine signal, therefore except this signal (δ 25.5), and four methyl signals (18–23 ppm) all the other 14C signals appeared downfield (δ 121–149) which verified the aromatic nature of the compound. The ^1H NMR spectrum there were two pairs *o*-coupled proton signals. The more downfield doublets, belonged to ring A protons with a coupling of 9 Hz, were appeared at δ 8.30 (H-1) and 7.60 (H-2) and another doublet pair belong to ring B with a coupling of 8.8 Hz were resonated at δ 7.85 (H-6) and 7.40 while two singlets appeared at δ 7.91 and 7.57 for H-11 and H-14, respectively. The UV data with the bands at 452, 360, 340, 308, 260 and 220 nm supported this structure. The unambiguous assignment of the entire structure was possible by a COLOC experiment. All spectral data together enabled the structure 12-hydroxyabieta-1,3,5(10),6,8,11,13-heptaen, named 12-demethylmulticaulin, being demethyl derivative of multicaulin, isolated from the same plant extract. Besides the four new norabietanes multicaulin, demethylmulticaulin, multiorthoquinone and 12-demethylmultiorthoquinone, two new abietanes 12-methyl-5-dehydrohorminone and 12-methyl-5-dehydroacetylhorminone and a new pimarane diterpene salvipimarone were also isolated from *S. multicaulis* in this study [32].

Salvia species also afforded many triterpenes [10,31], however, their structures were not as much interesting and diverse as abietane diterpenes. Most of them have oleanane or ursane type skeleton, and some lupanes. In fact, some of highly hydroxylated triterpenes showed cytotoxic activity, and especially a few of them found to be highly active against some cell lines [10,33,34]. One of the most active triterpenes betulinic acid was also isolated from some Anatolian *Salvia* species. But, in this paper, we aimed to emphasize the rare and interesting constituents of Lamiaceae family plants, particularly their diterpenic constituents, investigated through intensive NMR analyses by our group.

3.1.2. Sideritis diterpenoids

Likewise *Salvia* (sage) (folkloric name is island tea) species, *Sideritis* species are also consumed as tea, particularly in rural area, and known as mountain tea or walley tea, replaced *Salvia* plants in preparing tea in Turkey. There are 46 *Sideritis* taxa growing in Turkey and endemism ratio is very high (78%). Until now, we have investigated 12 *Sideritis* species [35–38], however, some elucidation studies on them have not been completed yet. The most characteristic property of kaurane diterpenes compared to abietane diterpenes is the lack of an isopropyl group and to contain four cyclic rings instead of three rings present in abietanes. Kaurane/*ent*-kaurane ring system contains four methyl groups in the free form, however, one or two methyls in the form of alcohol, aldehyde or double bond are commonly found which can be distinguished by NMR spectra. In their ^1H NMR, methyl protons of ring A as singlets and H-13 can be recognized by a characteristic multiplet easily. In our isolated *ent*-kauranes from Anatolian *Sideritis* spe-

Table 2
^{13}C NMR spectral data of norabietanes isolated from Anatolian *Salvia* species

C	Salvirecognin	Salvi-recognone	Multicaulin	12-Demethyl multicaulin	Multiortho-quinone	2-Demethyl multi-orthoquinone	Kronen-quinone	Salvicanaric acid	Salvimultine
1	126.9	125.7	120.9	121.0	152.8	153.0	153.2	38.3	200.7
2	23.4	23.3	128.0	128.2	133.9	133.2	135.7	17.6	53.3
3	36.9	40.0	140.0	140.0	140.6	141.0	141.7	37.6	36.5
4	38.9	37.8	135.6	135.5	133.4	133.5	133.4	37.7	47.6
5	45.9	44.9	122.7	123.0	127.6	127.0	127.6	114.9	23.5
6	19.4	35.4	125.5	125.6	125.5	122.6	122.8	–	29.9
7	30.2	198.4	127.9	127.8	132.4	133.0	133.5	171.1	129.7
8	125.4	122.7	126.1	126.3	128.5	128.9	126.0	133.9	152.2
9	133.9	134.6	147.8	147.8	126.0	127.0	127.3	118.0	119.2
10	133.4	133.4	133.4	133.4	152.4	150.1	150.1	51.3	156.9
11	109.4	109.6	110.6	109.9	183.3	183.2	182.8	137.6	127.1
12	158.2	158.2	151.4	148.9	181.4	183.0	150.5	143.1	156.9
13	138.2	141.6	130.3	130.0	135.3	134.9	134.9	142.9	139.2
14	116.7	124.5	125.5	124.9	126.6	126.5	184.2	123.1	11.1
15	29.7	26.9	25.5	26.0	26.3	25.8	26.2	27.1	27.2
16	26.7	26.9	22.7	22.8	23.0	22.9	22.8	22.3	22.4
17	20.9	22.3	22.7	22.8	23.2	23.0	22.7	22.4	22.4
18	31.6	29.8	20.9	21.0	20.8	21.0	21.1	24.4	20.3
19	22.6	22.4	18.3	18.4	17.9	17.6	17.7	26.1	29.1
20			55.4 (OMe)		56.0 (OMe)			17.4	

cies, two methyls are observed, in general, while one of the other two methyls was converted to hydroxy methylene group. In the lack of hexocyclic methylene at C-16, either a double bond or an epoxy group would be present between C-15 and C-16, most probably. Kaurane diterpenes were not found extremely interesting compounds to investigate through various NMR techniques due to very similar spectral properties, at least this was an observation for our isolated kauranes up to date. Although, sometimes, very similar structures can be more problematic in elucidation of their structures than more distinct structures.

From *Sideritis* species, a few labdane, pimarane and a beyerane diterpenes have also been isolated during our studies [37,38]. Particularly kaurane and beyerane diterpenes showed very similar spectral properties due to high similarity of their structures. The formulae of most common *ent*-kaurane diterpenes from Anatolian *Sideritis* species are seen in Fig. 12.

Sideritis species are also rich in flavonoids and phenolic structures, but most of these structures can be isolated from more polar plant extracts rather than dichloromethane or acetone extracts which are going to be studied by us. Another important point in structure elucidation of phenolics, especially in flavonoids, not only NMR, but also UV data taken with UV shift reagents can be very informative and even necessary.

3.1.3. Teucrium diterpenoids

The other interesting diterpenes were obtained from *Teucrium* species which required enormous NMR experiments for their structure elucidation. *Teucrium* species have been used for more than 2000 years as medicinal plants, and there are about 300 *Teucrium* species in the world and 27 species growing in Anatolia. They are pronounced with their neo-clerodane diterpenes with a furane moiety, even these diterpenes are known taxonomic markers of these species.

Neo-clerodanes consist of three or four ring systems. In general, either a five-membered spiro-lactone or a lactol ring attached to ring B at C-9 which is considered as third ring (ring C) or the latter ring is missing, and an ethylene group is present. A furan ring, attached to either ring C or to ethylene group, is almost present to form third or fourth ring of the skeleton, the two α and one β-protons of furane ring usually resonate at δ 7.40–7.45 as narrow doublets and a ddd at δ 6.30–6.40. Occasionally, a six-membered pyrane ring is present instead of a five-membered ring C, but this type of neoclerodanes mostly found in *Salvia* species. In fact, we could not isolate any *neo*-clerodane diterpene from Turkish *Salvia* species. Up today, South American *Salvia* species have afforded neo-clerodanes, in general. While neo-clerodanes were isolated from the aerial parts of *Teucrium* species, rearranged abietane diterpenes were obtained from the roots of these species, named teuvincenones, and this series rearranged abietanes first isolated from *T. polium* by a Spanish-Italian group. Similar rearranged abietanes were also isolated from the

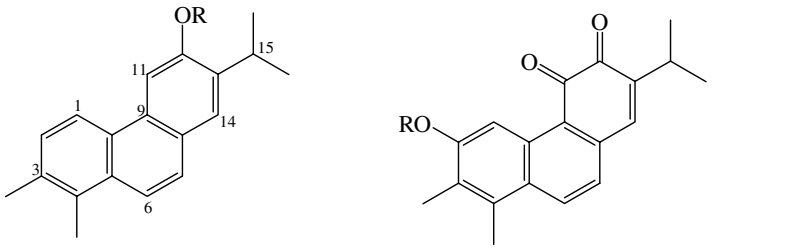


Fig. 11. Anti-tuberculosis nor-abietanes isolated from *Salvia multicaulis*.

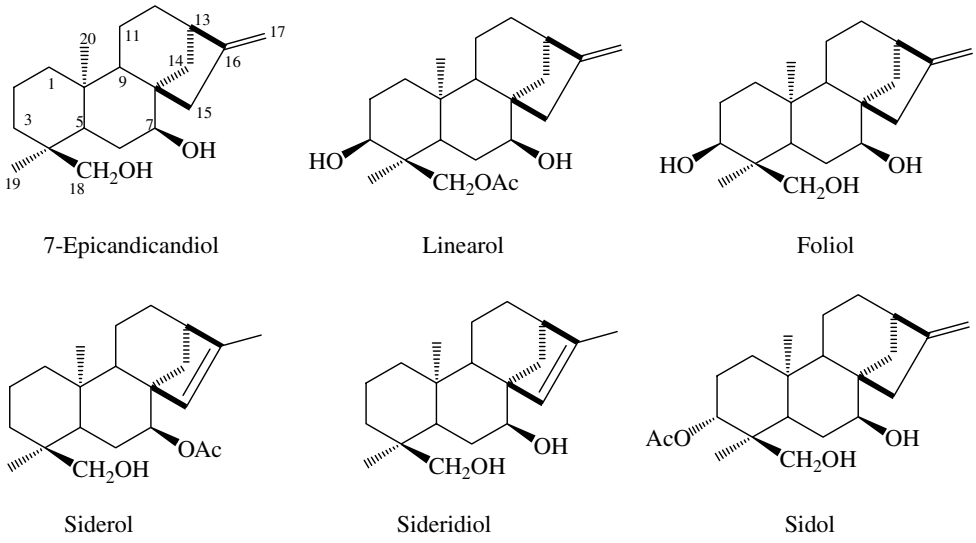


Fig. 12. Common ent-kauren diterpenes in *Sideritis* species.

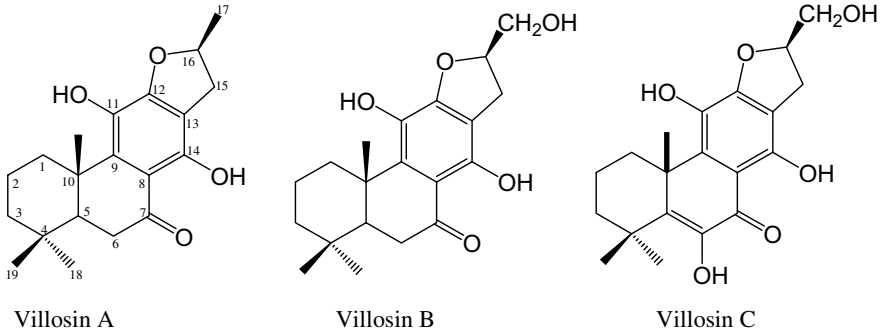


Fig. 13. Rearranged abietanes isolated from *Teucrium divaricatum* ssp. *villosum*.

roots of *T. divaricatum* ssp. *villosum* by our group [39]. One of the constituents of the latter plant was teuvincenone B which is a known rearranged abietane [40], the others were elucidated as new teuvincenone derivatives, which were named as villosins A, B and C, all the three have 17 (15 → 16)-*abeo*-abietane skeleton (Fig. 13). Their structures were established by using NMR and MS spectral data [39] (Table 3).

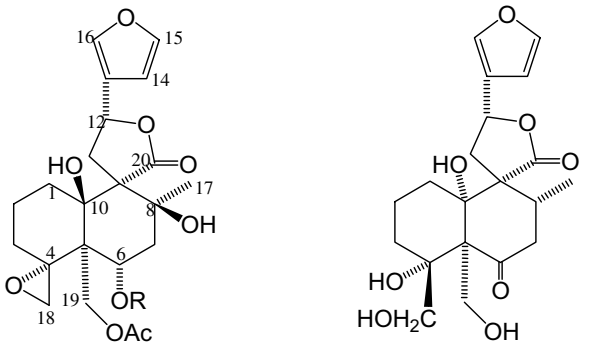
We have isolated very interesting neo-clerodanes from two species which were collected from Sandras mountain, Mugla, located in Aegean part of Turkey. Their structures were determined by intensive 1D and 2D NMR techniques.

One of Sandras mountain *Teucrium* species, *T. sandrasicum* afforded [41] two new C-10 β-hydroxylated and one α-hydroxylated neo-clerodane diterpenes, named sandrasin

Table 3
^{13}C NMR spectral data of diterpenoids isolated from Anatolian *Teucrium* species

C	Villosin A	Villosin B	Villosin C	6-Deacetyl sandrasin A	Sandrasin B	Sandrasin C	Alysine A	Alysine B	3-Deacetyl alysine B	Alysine C	Alysine D	Alysine E
1	36.4	27.5	27.8	31.8	30.6	25.4	65.00	64.96	65.00	18.64	18.6	18.6
2	16.2	19.0	19.0	18.8	18.9	14.9	40.60	37.86	39.78	31.63	32.9	32.9
3	41.1	40.5	41.0	29.4	29.3	21.0	74.60	71.28	74.31	74.00	75.1	75.3
4	37.2	36.4	37.0	62.9	63.9	74.8	63.27	71.37	76.99	66.24	62.3	75.1
5	49.9	49.2	49.5	49.8	51.6	51.9	45.60	44.79	45.15	44.06	42.1	41.8
6	29.7	32.9	140.1	67.3	64.9	197.0	75.40	75.93	74.31	74.00	75.4	75.4
7	185.6	185.3	182.0	38.2	39.3	37.7	206.70	206.83	206.41	207.38	106.6	106.6
8	107.6	107.8	108.0	75.8	76.0	31.2	53.91	53.49	53.71	50.89	51.6	51.6
9	139.5	138.5	138.4	59.3	59.6	53.8	49.20	52.44	52.70	49.53	48.1	48.1
10	35.4	36.0	39.6	81.9	81.5	84.0	45.73	42.02	42.47	41.96	42.1	41.8
11	131.1	131.1	131.0	34.8	34.9	33.0	19.30	18.94	19.32	17.23	18.6	18.6
12	155.6	154.0	153.3	72.2	72.1	71.2	33.62	34.45	34.92	32.37	29.1	29.6
13	110.6	110.5	110.6	124.9	125.0	119.2	116.30	116.54	116.61	115.41	115.7	115.7
14	154.8	154.7	154.6	108.0	108.0	107.3	151.90	151.21	152.36	151.84	154.1	154.2
15	34.3	35.4	35.4	144.2	144.1	143.2	110.44	110.25	110.47	110.42	109.5	109.1
16	83.2	82.9	83.2	139.6	139.6	139.4	142.50	142.45	142.74	142.57	141.9	141.9
17	24.5	65.0	65.1	26.0	26.1	15.3	19.19	18.08	18.57	18.44	17.1	17.2
18	33.2	33.0	27.7	51.6	51.6	69.8	49.20	66.21	66.48	50.89	49.9	66.4
19	21.6	21.5	21.0	63.6	63.8	59.2	62.90	65.50	60.63	62.63	66.7	66.4
20	16.2	17.2	25.2	174.6	174.8	174.2	17.82	17.49	17.72	18.90	20.2	20.2
O–$COCH_3$[a]				170.6, 21.2	170.8, 21.1	–	170.1, 20.7	170.0, 20.2	170.1, 20.6	169..8, 20.7	169..6, 21.2	170.6, 21.2
O–$COCH_3$[a]				170.6, 21.2	–	–	169.7, 20.9	169.6, 20.5	169.8, 20.8	–	–	171.2, 20.2
O–$COCH_3$[a]				–	–	–	–	169.6, 20.8	–	–	–	–

[a] The ^{13}C chemical shift values of the carbonyl and methyl signals' which belong to acetoxy groups are given in only one decimal point due to limited room in the cells, the others are given according to their original published data.



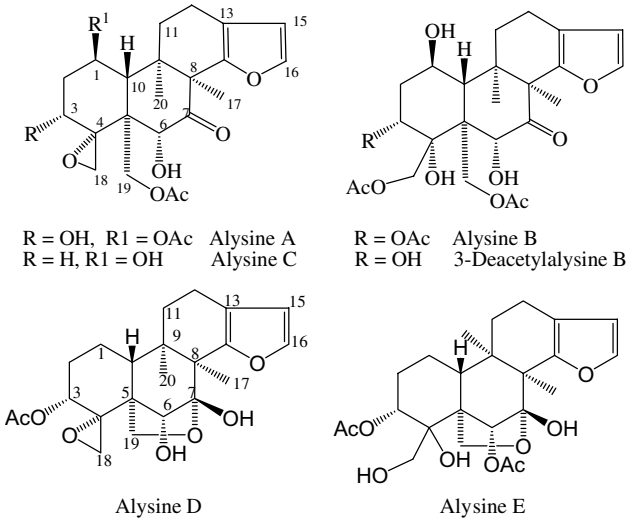
R = Ac Sandrasin A
R = H 6-Deacetylsandrasin A

Sandrasin B

Fig. 14. Neo-clerodane diterpenoids from *Teucrium sandrasicum*.

A, 6-deacetylsandrasin A and sandrasin B (Fig. 14), all three compounds showed C-12 R configuration. This configuration was deduced by the observation of a NOE between Me-17 and H-12. Because stereochemical considerations showed that *Teucrium* diterpenoids with the C-12 R configuration have their Me-17 group and H-12 which are nearly parallel steric disposition on the same side of the lactone ring. The presence of a hydroxyl group at C-10 was deduced by paramagnetic chemical shifts of C-1, C-5 and C-9 protons. The α-configuration of hydroxyl group at C-10 followed from the NOE correlation between C-10 α-OH and C-19 protons.

Another Sandras mountain *Teucrium* species is *T. alyssifolium*, its acetone extract of the aerial parts afforded six new *neo*-clerodane diterpenes [42,43]. Since the two of isolated *neo*-clerodanes (alysines A ad B) gave nice crystals, their single crystal X-ray analyses (Fig. 16) were also carried out [42] which led to determine their novel skeleton with their stereochemistry besides by extensive NMR analyses.



R = OH, R1 = OAc Alysine A
R = H, R1 = OH Alysine C

R = OAc Alysine B
R = OH 3-Deacetylalysine B

Alysine D

Alysine E

Fig. 15. Neo-clerodanes with α,β-disubstituted furan ring from *Teucrium alyssifolium*.

All six compounds had typical substitutions of neo-clerodane diterpenes (Fig. 15), however, they all have α,β-disubstituted furan ring, instead of monosubstituted one, therefore the linkage between ring C and furan ring was different than those of other neo-clerodanes.

The ^{13}C NMR (BB; APT and DEPT) revealed four methyl, five methylene, six methine and nine quaternary carbon signals for 24 C atoms (Table 3). The signals at δ 206.70, 170.13 and 169.68 were belonged to carbonyl signals, the last two being acetate carbonyls. The HRMS exhibited a molecular formula $C_{24}H_{30}O_9$ corresponding to a molecular ion peak at *m/z* 462.1884.

The ^{1}H NMR spectrum of alysine A showed two tertiary methyl singlets at δ 1.14 (Me-20) and 1.34 (Me-17) and two acetyl group methyls at δ 2.02 and 2.06. The presence of an α,β-disubstituted furan ring was observed with the signals at δ 7.37 and 6.24 with 2 Hz of vicinal coupling. A pair of upperfield oxymethylene protons as narrow doublets at δ 3.08 and 2.62 was indicative of a spiro-oxirane ring at C-4 (^{13}C signal at δ 49.20) which was typical location for neo-clerodane diterpenoids. The second pair of oxymethylene protons were observed in more downfield at δ 4.27 and 4.68 with a coupling constant of 12 Hz attributing to C-19 protons (^{13}C signal at δ 62.90). There were also three oxygenated methine protons at δ 4.45, 4.59, and 3.80, their location followed from spin-decoupling, ^{13}C NMR and HETCOR experiments (Fig. 17) and deduced that the first attached to C-1 (δ 65.00) and the last to C-6 (75.40), and both next to hydroxyl group, while the other one (δ 4.59) located at C-3 (δ 74.60) next to an acetyl group. Their chemical shifts and geminal couplings also indicated their stereochemistry in these centers. The presence of the acetyl groups in the structure was verified by IR, the oxo group, as well. The location of the oxo group at C-7 followed by selective INEPT experiments which is fairly rare in neo-clerodanes. By spin decoupling experiments, the sequence C_{10}–C_3, and other relations between protons were observed and determined. Unambiguous assignment of the protons and carbons including the location of α,β-disubstituted furan ring in the skeleton followed from HETCOR, HMBC and SINEPT experiments and confirmed by X-ray analysis (Fig. 16). Thus, neo-clerodanes, having α,β-disubstituted furan ring, have been isolated for the first time from Anatolian *Teucrium* species.

By X-ray analysis, chair conformation of both A and B rings and their cis fusion were determined (Fig. 16). The X-ray analysis also exhibited cis fusion of ring C to the ring B, and half-chair conformation of ring C which was fused to an α,β-disubstituted furan ring. Intramolecular H bond between keto group at C-7 and oxygen (O5), and intermolecular H bond between O1 and carbonyl oxygen O8. In alysine B, (Fig. 15) the FAB and HRMS indicated a molecular formula $C_{26}H_{34}O_{11}$ which had a very similar structure to alysine A, the only difference was observed at C-4. Because, the spiro-oxirane ring signals were missing in alysine B, instead a downfield methylene pair signals were observed at δ 4.06 and 4.22 with a geminal coupling of

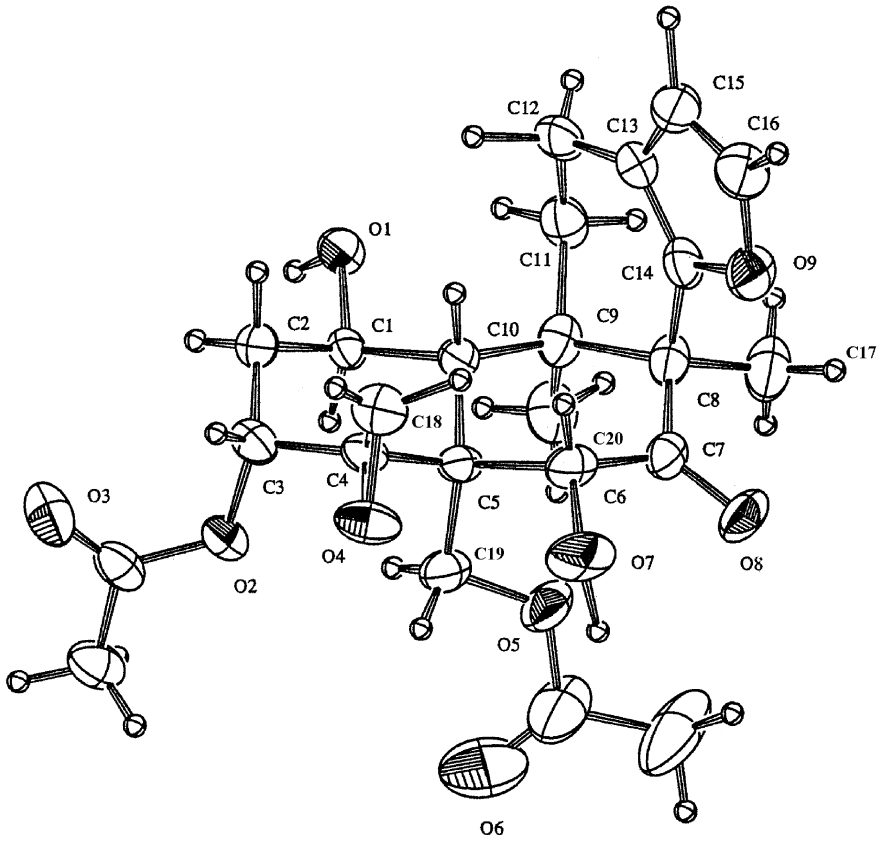


Fig. 16. X-ray structure of Alysine A.

12.5 Hz. Considering all the other spectral data, it was deduced that these two proton signals at C-4 belong to an acetoxymethylene protons and next to a hydroxyl group. Other part of the structure was exactly the same with alysine A. The other two same type α,β-disubstituted furan ring containing new neo-clerodanes were determined through NMR, mass, IR and UV spectroscopic analyses [42] and named as 3-deacetylalysine B and alysine C. From *T. alyssifolium* isolated other two new neo-clerodanes, named alysines D and E (Fig. 15) [43], had a hemiacetal group between C-19 and C-7, in these compounds a signal at δ 106.6 was very significant for the hemiacetalic carbon (C-7).

3.1.4. Ajuga diterpeonids

Since both *Teucrium* and *Ajuga* species are known with their anti-feedant and insecticidal properties and these properties are attributed to their neo-clerodane skeleton we aimed to investigate *Ajuga* species along with *Teucrium* species. However, the constituents were not found to be very similar in Anatolian examples investigated by us. In general, *Ajuga* species contain steroids, particularly ecdysteroids, iridoids, and polyphenolics besides neo-clerodanes. In fact, we have isolated some triterpenoids and steroids rather than ecdysteroids and diterpenes from Anatolian *Ajuga* species. The isolated two diterpenes from *Ajuga chamaepitys* ssp. *laevigata* [44] are shown in Fig. 18, only one new diterpene, named ajugalaevigatic acid, has a clerodane structure while the other has a labdane structure [(13S)-15-hydroxylabd-8(17)-en-19-oic acid]. Compared to *Teucrium* species, studied *Ajuga* species growing in Anatolia were found to be more rich in steroids [44,45] and iridoids [46].

In fact, some neo-clerodanes isolated from *Teucrium* species have been searched for their insect anti-feedant activity, but they have not exhibited any meaningful activity. In addition, some *Sideritis* extracts and their constituents have been searched for their anti-feedant and insecticidal properties, and showed more or less activity against some stored pests [47].

3.1.5. Nepeta iridoids

The other Lamiaceae family member *Nepeta* genus is represented over 250 species in the world and 33 are found in Turkey. Most of the species were investigated for volatile constituents. *N. caesarea* [48] was investigated for involatile secondary metabolites by our group and its extract afforded four new compounds which formed a conjugation between an iridoid and a triterpenoid. In fact, in literature, there are some conjugated compounds which formed iridoids and diterpenoids, however, this was the first example of iridoid-triterpene conjugated structures in *Nepeta* species. We have also investigated four *Nepeta* species for volatile constituents (essential oils) by GC–MS analysis [49].

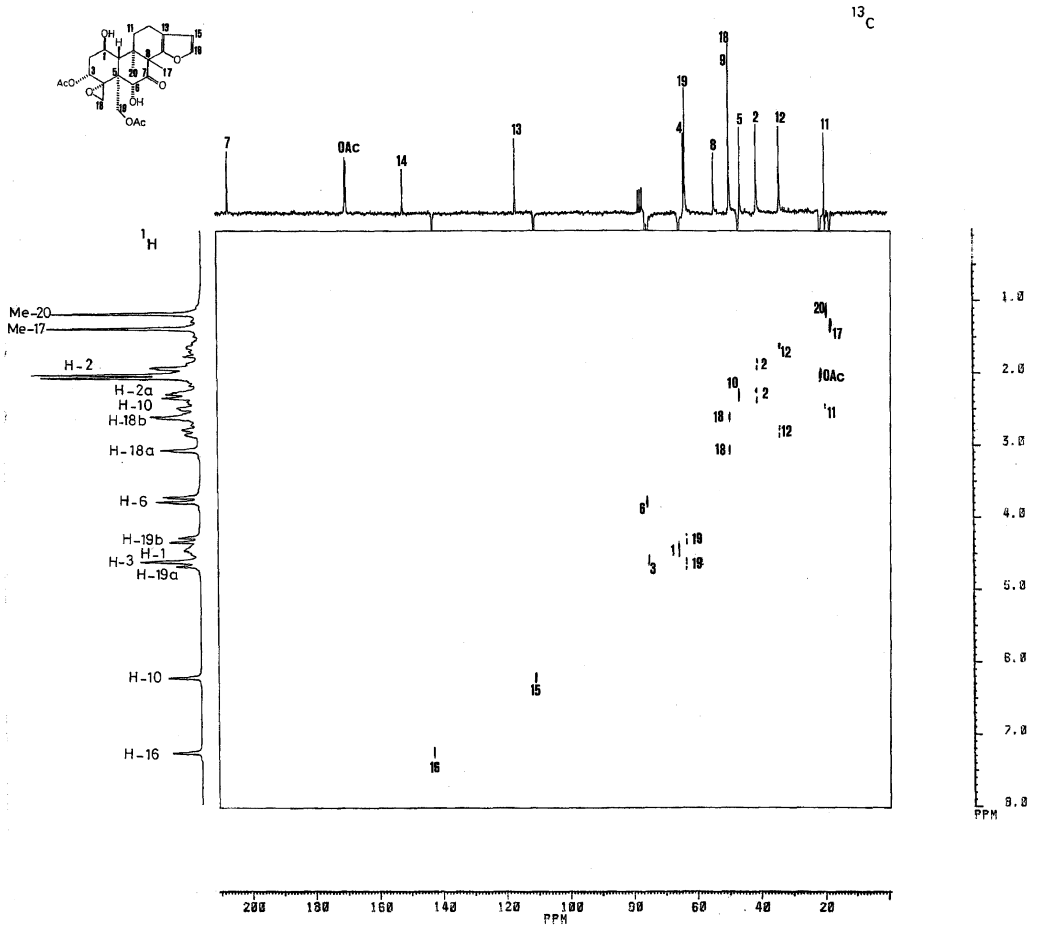
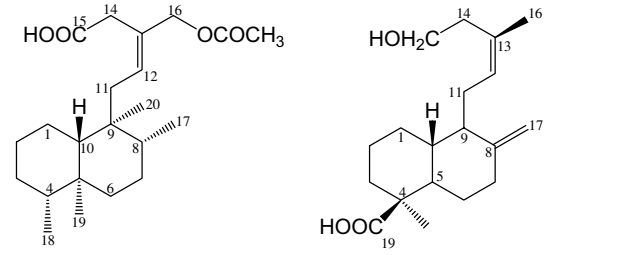


Fig. 17. HETCOR experiment of Alysine A.



Ajugalaevigatic acid 13(S)-15-Hydroxylabd-8(17)-en-19-oic acid

Fig. 18. Two diterpenoids of *Ajuga chamaepitys* ssp. *laevigata.*

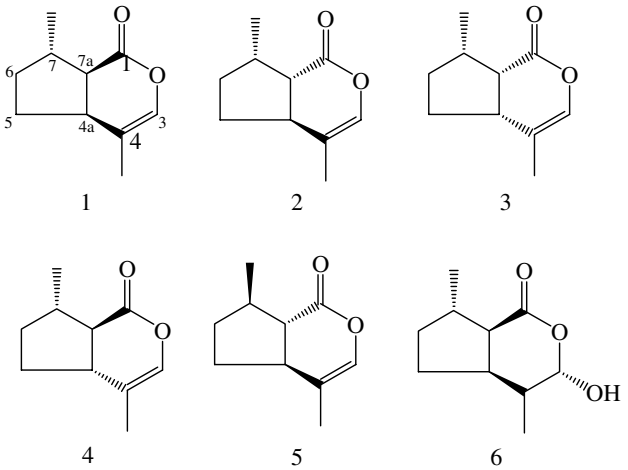


Fig. 19. Nepetalactones with iridoid structure isolated from *Nepeta nuda* L. ssp. *albiflora.*

Nepetalactones are characteristic iridoid monoterpenes for *Nepeta* species as volatile constituents and are important compounds with insect repellant and feline attractant properties. We have isolated a series nepetalactones from both essential oil and the hexane extract of *N. nuda* L. ssp. *albiflora* (Boiss.) Gams. (Fig. 19) [50]. Their structures were elucidated with stereochemistry only by separate isolation of each nepetalactone, therefore, 6 pure stereoisomers of nepetalactones from the hexane extract of *N. nuda* L. *albiflora* [50] were isolated, and the structures were elucidated. Since all have the same molecular formula $C_{10}H_{16}O_3$ and molecular ion at *m/z* 166, except nepetalic acid (**6**) (3α-hydroxy-4aα,7α,7aα-dihydronepetalactone), their ^{1}H NMR spectra are very informative to distinguish them, particularly H-3, Me-8 and Me-9 signals were differentiated in determining the structures [50].

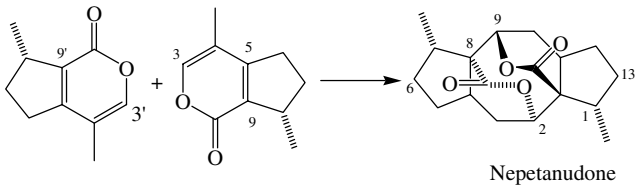


Fig. 20. Possible formation of dimeric nepetalactone (nepetanudone) isolated from *Nepeta nuda*.

Furthermore, a dimeric nepetalactone, trivial name nepetanudone (Fig. 20), was isolated from the hexane extract, as well as the acetone extract of the same plant along with some known triterpenes. Its structure has been previously determined [51] by isolating from *Nepeta tuberosa* ssp. *tuberosa*, however, it was verified by X-ray analysis (Fig. 21) by our group [52]. This compound should be considered to arise from [4 + 4] cycloaddition of two 5,9-dehyronepetalactone moieties. If the stereochemistry was not present at the C-7 and C-14 positions, the molecule could contain a center of symmetry, however, there is no symmetry element relating to two halves of the molecule and the NMR showed a doubling of each resonance (Fig. 21).

In structure elucidation of iridoid-triterpenoid or iridoid–steroid conjugated structures (Fig. 22) isolated from *Nepeta caesarea*, HREIMS and/or FABMS (+) played important role besides NMR spectroscopy [48]. In both steroid conjugated iridoids, molecular peak was observed at *m/z* 580, and followed by the loss of iridoid (dihydronepetalactone) unit with ether linkage [$C_{10}H_{15}O_3$] at *m/z* 396 as a base peak. Their NMR spectra were also very similar, the main difference was observed for the olefinic proton and C-3′ methine signals. The *J* value and chemical shift difference of H-3′ signal indicated opposite stereochemistry at C-3 in these two structures.

Two iridoid-triterpenoid structures also exhibited similar mass fragmentation patterns. The oleanane derivative one, 3′α-[olean-12-ene-28-oyl-3β-oxy]dihydro- nepetalactone, exhibited fragment ions at *m/z* 454 and 439 indicating the loss of dihydronepetalactone moiety which was observed as a base peak at *m/z* 167. The lupane derivative 3′α-[lup-20(29)-ene-28-ol-3β-oxy]dihydronepetalactone exhibited a vinylic methyl at δ 1.68 and a pair of methylene protons at δ 4.68 and 4.59 in ^{1}H NMR besides five methyl and other signals indicating its lupen skeleton which gave a molecular ion peak at *m/z* 608. Other informative fragment ions about the structure appeared at *m/z* 577 [$M-CH_2OH]^+$, 453, 440 [$M-C_{10}H_{15}O_2$], 167 (dihydronepetalactone) verifying the conjugated structure [48].

3.1.6. Lavandula triterpenoids

Lavandula genus is only represented by two species in Turkey. One species, *L. angustifolia* Miller ssp. *angustifolia* Miller, and the second *L. stoechas* L., the latter has two subspecies; ssp. *stoechas* L., and ssp. *cariensis* (Boiss.) Rozeira. In an earlier study on Turkish *Lavandula stoechas* ssp. *stoechas* longipinene derivatives, one being new, were obtained besides some known terpenes [53]. In a later study, the roots of the same species afforded eleven known triterpenes with oleanane, ursane and lupane skeleton [54]. In addition, two steroids and two aromatics, and two new triterpenes, 18-hydroxy-27-norolean-12,14-dien-30-al-28-oic acid and 3β-hydroxy-1-oxo-olean-12-ene-30-al-28-oic

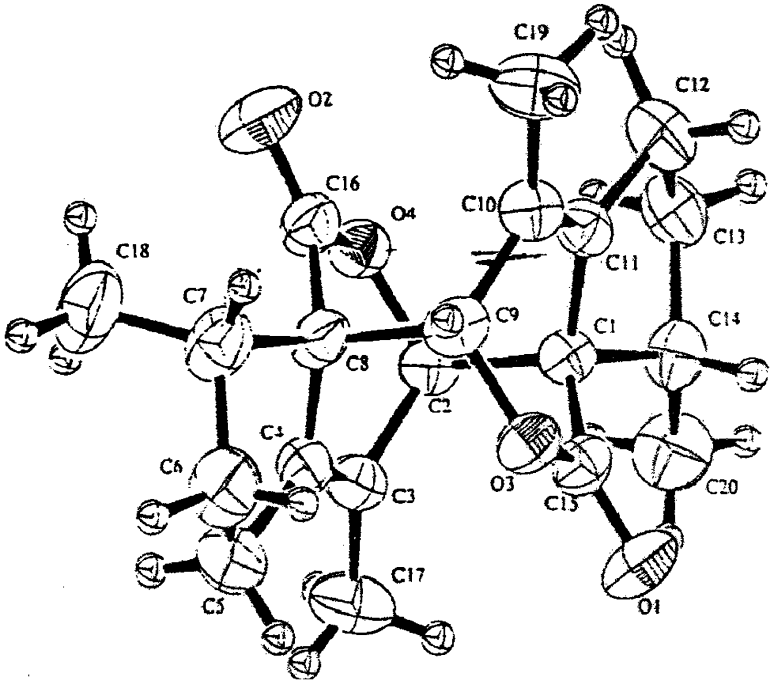
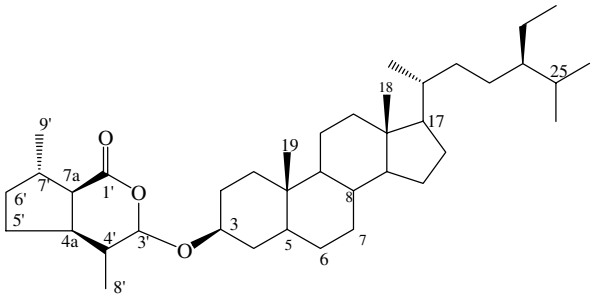
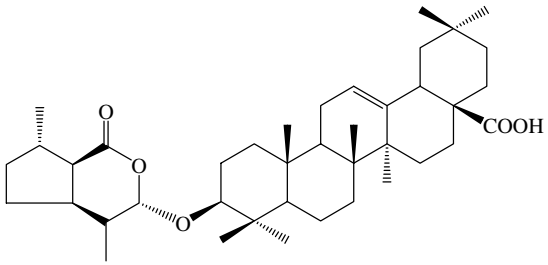


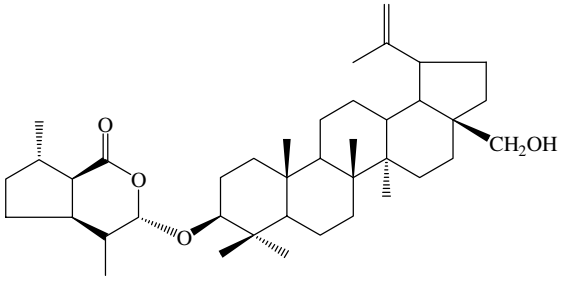
Fig. 21. The X-ray structure of nepetanudone.



Δ^5 3'-[β-sitosteryl-3-oxy]dihydronepetalactone
Δ^7 3'-[5-stigmasta-7-ene-3-oxy]dihydronepetalactone



3'-[olean-12-ene-28-oyl-3-oxy]dihydronepetalactone



3'-[lup-20(29)-ene-28-ol-3-oxy]dihydronepetalactone

Fig. 22. The iridoid-triterpene constituents of *Nepeta caesera*.

acid have been isolated, and their structures were determined by spectroscopic analyses [54]. We also investigated essential oil of this species together with bioactivity [55].

4. Conclusions

Lamiaceae (= Labiatae) family plants are rich in diterpenoids. Among investigated Lamiaceae family plants growing in Anatolia, *Salvia* species showed highest diversity [8–10], particularly in diterpenes (abietane, rearranged and nor-abietanes) [18–21,25,26,31,32]. *Sideritis* species were rich in diterpenes [56], but only in kauranes [35–38,57], however, *Sideritis* kaurane diterpenoids were not shown diversity as much as *Salvia* abietane diterpenes.

No many *Teucrium* and *Ajuga* species have yet investigated chemically which grown in Turkey. However, *Teucrium* species showed very interesting and diverse neoclerodanes [57,58] as well as some rearranged abietanes while *Ajuga* species were rich in steroids [45,46] rather than diterpenoids.

Although diterpenes were not isolated from Anatolian *Nepeta* species, nepetalactones (as monoterpene iridoid) [49,50] and their conjugated constituents including one dimeric nepetalactone (nepetanudone) were found in our studied *Nepeta* species [48], and *Lavandula* species were rich in triterpenoids [53,54].

In the structure elucidation of all isolated secondary metabolites, modern one and two dimensional NMR techniques were used intensively. For 1D NMR measurements, ^{1}H, ^{13}C (BB, APT, DEPT), NOE, and spin-decoupling experiments between homo- and heteronuclei were carried out, such as direct ^{1}H–^{1}H-spin decoupling and three bonds away ^{1}H–^{13}C-spin decoupling experiments (selective INEPT) were carried out. For 2D NMR measurements, ^{1}H–^{1}H (COSY, NOESY) and ^{1}H–^{13}C correlation spectroscopy [(HETCOR, COLOC and HSQC), and in inverse probe HMQC, HSQC, and HMBC] experiments were

run. In addition, IR, UV and mass (EI-or CI- or FAB-Mass) spectral measurements, and for the new compounds, HRMS analyses were run. When we get proper crystals, X-ray analyses were also performed.

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