CONSTITUENTS OF TWO BALLOTA SPECIES FROM IRAN; BALLOTA AUCHERI AND BALLOTA NIGRA.

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ABSTRACT
Ballota (horehound) is a genus of flowering evergreen perennial plants and subshrubs in the family Lamiaceae. Native to temperate regions. The Mediterranean region has the highest diversity in the genus, with more isolated locations in South Africa, Central Asia, northern Europe, and the islands of the eastern North Atlantic. It is found in rocky and waste ground.

KEYWORD: Iranian Ballota Species (Ballota aucheri and Ballota nigra), Labiatae, Stachydiodeae.

INTRODUCTION
From the genus Ballota (Labiatae, tribe stachydiodeae) already several species have been studied chemically. Typical compounds found are furanolabdanes which have been reported from seven species.1-8 We now report the results of our studies on Ballota aucheri Boiss. and Ballota nigra L.

Ballota Aucheri Boiss
The extract of the aerial parts of Ballota aucheri afforded in addition to ballotinone 1[10], the diterpenes 2, 3, 4a-4c and 5.[9]

The 1HNMR spectrum of 2 indicated the presence of a furanolabdane. Thus it contained the typical signals of a β-substituted furan at δ6.29 ppm, 7.25 ppm and 7.38 ppm, and methyl singlets at δ1.31, 0.98 and 0.91 ppm together with a methyl doublet at δ1.27 ppm. A doublet at δ3.89 ppm was coupled with a double quartet at δ1.88 ppm, which itself showed a vicinal coupling with the methyl doublet. Accordingly, a 7-hydroxy derivative was indicated. The absence of a further coupling of H-7 and the downfield shift of a slightly broadened singlet at δ2.95 required a keto group at C-6. All these data, therefore, correspond to the proposed structure 2. The coupling J1,6 indicated the configuration at C-7 and C-8. The structure was further supported by the 13CNMR spectrum. Compound 2 is closely related to hispanolone[4] which, however, has a 7-keto group and no function at C-6. Of course, the NMR data are in part similar.[9]

The 1HNMR spectrum of 3 showed some similarities to that of 2. However, the typical furan signals were replaced by pairs of doublets (66.49 and 5.22, 4.56 and 4.14 ppm) typical for dihydروفuran. The stereochemistry of 3 was deduced from the observed NOES [H-20 with H-19 (5%) and H-11 (6%), H-18 with H-5 (7%), H-17 with H-16 (3%) and H-7 with H-5 (4%)]. The 13CNMR data further support the structure of 3 which is presumably formed by stereospecific addition of the 9-hydroxy group at the 13(16)-double bond. This may be an indication that the ketone 3 is not an artifact though formation of such ethers has been observed on acid treatment of precursors like 2. However, the stereochemistry of the addition was not determined.[10, 2]. The 13CNMR data also agree with the proposed structure.[9]

The 1HNMR spectra of 4a-e differed markedly from those of 2 and 3. In the case of 4a spin decoupling clearly showed that a hydrogen bonded 6-hydroxy-7-keto derivative was present. The small coupling J3,6 required a 6β-hydroxy group. The downfield shift of H-8 (δ3.45) required a 7-keto group. A clear doublet at δ1.96 was due to the hydroxy proton. The lowfield signal at δ4.93 showed vicinal couplings with a pair of doublets at δ3.45 and 3.54 ppm together with a methyl doublet at δ3.7 ppm. A pair of doublets at δ7.15 and 2.08 which showed some similarities to those of 2 and 3. In the case of 4a spin decoupling clearly showed that a hydrogen bonded 6-hydroxy-7-keto derivative was present. The small coupling J3,6 required a 6β-hydroxy group. The downfield shift of H-8 (δ3.45) required a 7-keto group. A clear doublet at δ1.96 was due to the hydroxy proton. The lowfield signal at δ4.93 showed vicinal couplings with a pair of doublets at δ3.45 and 3.54 ppm together with a methyl doublet at δ3.7 ppm. A pair of doublets at δ7.15 and 2.08

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for compound 4c indicated that it was the corresponding ethoxy derivative of 4s. It is likely that compounds 4a-c is formed by addition of methanol and ethanol, respectively, to the corresponding dihydrofuran, e.g. 3. However, the required 7-keto isomers of 3 or 2 were not isolated. The 1H NMR spectrum of 5 indicated that again a β-substituted furan was present which, however, had to be very different from 2. A methyl singlet at δ2.00 had to be assigned to an olefinic methyl rather than to an acetate methyl as the molecular formula for 2 was C20H26O3. A low field singlet at δ6.99 appeared to be due to an α-proton of an enolest. The 13C NMR spectrum supported this idea. All data agreed with the presence of the enollactone 5. In particular the singlets at δ165.8 and 181.7 supports the conjugated lactone grouping as the C-9 signal should be strongly downfield shifted due to the electron withdrawing effect of the carbonyl group and the high degree of substitution. The lactone 5, which we have named Balloaucherolide, must be formed by oxidative degradation of a precursor of 4a-c like 6 which could be formed by elimination of water followed by oxidative cleavage of the 6,7-bond and formation of the enollactone (Scheme).[9]

The additional results on the chemistry of Ballota show that diterpenes derived from furanolabdanes are characteristic of, but not confined to the genus as similar compounds have been isolated from other parts of the tribe Stachydoideae, e.g. Marrubium, Galeopsis and Salvia, all placed in the same sub tribe.[11]

The 1H NMR spectrum of 8 showed signals typical of a β-substituted furan ring, and four tertiary methyl signals pointed to a labdane skeleton. A doublet of doublet at δ4.34 (H-6), which was coupled with a methine doublet at δ1.54 (H-5) and a D2O-exchangeable signal at δ3.32 supported an axially oriented secondary hydroxyl group. That ring A was not functionalized was indicated by three consecutive methylene groups. The 13C NMR spectrum contained signals for a double bond and a keto group. The chemical shift of the latter was in accordance with a conjugated ketone. Thus, the structure 8 was likely and final confirmation was achieved by 2D hetero and homo COSY experiments. NOE difference spectroscopy was also helpful for assignment of methyl signals. Important MOEs were observed between H-18, H-19, H-5 (7%) and H-6 (12%) as well as between OH, H-6 (5%) and both axial methyl groups H-19 and H-20.[13]

The 1H NMR spectrum of 4 was in part similar to that of 3. Again a β-substituted furan ring and the sequenced H-1 _ H-3 and H-11 _ H-12 were present, but now only three tertiary methyl signals were observed. The most
striking signals were those for an ethyl group, a structural feature not very common among diterpenes. Further information came from the \(^{13}\)CNMR spectrum, which showed a keto group and a singlet at δ108.0 suggestive of an acetal carbon.\(^{13}\) Furthermore, a doublet at δ81.8 required an additional oxygen-bearing tertiary carbon. A corresponding signal in the \(^1\)HNMR spectrum at δ4.25 appeared as a doublet and was coupled with a singlet at δ2.29 (H-5). A sharp singlet at δ3.01 was due to a tertiary hydroxyl group. Taking into account all of these facts the structure 9 was likely. The stereochemistry followed from the results of NOE experiments. Effects were observed between H-18, H-5 (7%), H-3α (5%), H-3β (3%), H-8 (1.5%), H-6 (2%) and H-19, between H-19/20 and H-18, H-2, H-11 and H-6 (13%) and between OH and H-5 (5%). The large coupling \(J_{5,6\beta} = 11.5\) Hz supported the trans-diaxial orientation of the corresponding hydrogens. The formation of this unusual compound could be easily explained in term of a retro aldol reaction starting from a derivative of \(\mathbf{1}\) having a tertiary hydroxyl group at C-9 and subsequent formation of the hemiacetal between a newly formed keto group at C-9 and the hydroxyl group at C-6.\(^{13}\)

The \(^1\)HNMR spectrum of compound 5 showed two sets of signals indicating a dimeric structure. By a combination of 2D homo and hetero COSY experiments, the positions of all the signals were localized and assigned to two furolabdane subunits. The H- and \(^{13}\)CNMR signals for the subunit A (Scheme 7) resembled those of compound \(\mathbf{2}\).\(^{9}\) However, several of the assignments of \(^{13}\)CNMR signals have to be corrected \(^9\). The difference in the chemical shifts of C-7 in compounds 2 and 10 (12.1 ppm) indicated that the connecting carbons in the dimer were probably joined by an ether linkage. The second subunit (B) gave rise to \(^{13}\)CNMR signals for a tetrasubstituted double bond, a keto group and an oxygen-bearing carbon, the latter being the coupling position to the subunit (A). As both H-5 and H-7 appeared as singlets, the keto group had to be placed at C-7. Thus, an ether bond between C-7 of both subunits was likely. This assumption was confirmed by a 2D long range hetero correlation experiment. The most important correlation was observed between H-7 of the subunit (A) and C-7 of the subunit (B). The stereochemistry was deduced from the results of NOE difference spectroscopy. The diaxial orientation of H-7 and H-8 (subunit A) was already indicated by the coupling \(J_{7,8} = 11.5\) Hz and further supported by NOE effects between H-7 and H-5, H-5 and H-8, as well as between H-20 and H-8. More problematic was the stereochemistry at C-7 of the subunit (B). A weak NOE effect between H-20 and H-5 indicated a 7α-oxygen function. The final confirmation was achieved after LiAlH\(_4\) reduction and formation of two isomers. The couplings in the ring B of the subunit (A) indicated the same stereochemistry in both isomers with a 6β-hydroxy group, obviously formed by hydride attack from the less hindered α-side. In the subunit (B) of the main product, the couplings indicated a 6β-hydroxy group while in the minor compound the large coupling \(J_{5,6\beta} = 12\) Hz indicated a 6β-hydroxy group. On the other hand, in both compounds the coupling \(J_{5,7}\) in the subunit (B) was small, indicating the axial orientation of the substituent at C-7. The stereochemistry was confirmed by the NOE results. In particular, the strong effects of both axial methyl groups (C-19 and C-20) and H-6 in 10b are important. The compounds were formed in the ratio 3:1.\(^{13}\)

The chemical shifts of H-7 in the natural compound and both reduction products, in particular those of the subunit (B), are worthy of note. Usually, an allylic proton in a \(\alpha\)-position to a keto group on an oxygen-bearing carbon is shifted downfield. The shielding cone of the carbonyl group in the counterpart could be responsible for the up field shift in the natural compound. In the reduction product, the \(\alpha\)-effect is missing causing an up field shift, but the shielding effect of the counterpart is no longer present. The strong NOE effect between certain signals of the different subunits indicated a preferred conformation. Similar effects were observed in the spectra of the reduction products. The conformational behavior and synthesis of model compounds are presently under investigation. We have named compound 10 Persianone.\(^{13}\)
**Ballota nigra L.**

The aerial parts of *Ballota nigra* was collected from province of Golestan, North of Iran. The composition of the essential oil of *Ballota nigra* was investigated by means of gas chromatography (GC) and GC-mass spectrometry (MS).

The essential oil of *Ballota nigra* contained β-pinene (39.0%) and α-pinene (34.5%) among the 12 constituents characterized, comprising 99.3% of the total components detected. Monoterpenes (96.5%) predominated over sesquiterpenes (2.8%). Other major components in this oil were sabinene (7.7%), α-phellandrene (4.1%), β-caryophyllene (2.8%) and 1,8-cineole (2.6%).\(^\text{[14]}\)

In 2007, the essential oil composition of *Ballota nigra* collected from the suburb of Nour, Mazandaran Province, north of Iran was investigated and a large amount of sesquiterpenes have been reported with caryophyllene oxide (7.9%), epi-muurolol (6.6%), δ-cadinene (6.5%) and α-cadinol (6.3%) as the main constituents.\(^\text{[15]}\)

The essential oils of *B. nigra* and *B. undulate* collected from Amman have been reported before. In the oil of two *Ballota* species sesquiterpenes were present in large amounts and germacrene D was the main component. In the same report *B. saxatillis* from Italy, showed monoterpenes much more than sesquiterpenes and the main compound was linalool.\(^\text{[16]}\) The chemical composition of essential oils obtained from the roots, stems and leaves of *Ballota nigra*, growing in Serbia was investigated, the plant produces two types of essential oils. Oils derived from stems and leaves were sesquiterpene rich, in contrast oil derived from the root was rich in oxygenated monoterpenes.\(^\text{[17]}\)

Essential oil of *Ballota aucheri* from Iran was characterized by a large amount of sesquiterpenes with α-cadinol (21.0%), dehydrotamandran (11.8%) and β-caryophyllene (8.1%) as the major constituents found.\(^\text{[18]}\) From the 52 identified constituents of the essential oil of *B. pseudodictamnus* caryophyllene oxide, phytol and γ-murolene were the major components; furthermore the essential oil was investigated for its antimicrobial activity.\(^\text{[19]}\)

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**Reference**

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