Nepetaefolinol and Two Related Diterpenoids from *Leonotis nepetaefolia*

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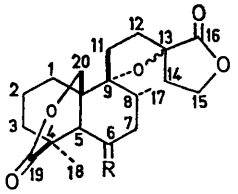
Nepetaefolinol, a new diterpenoid from L. *nepetaefolia*, is identified as 9,13-epoxy-6β-hydroxy-8α-labdane-16,15:19,20-diolactone (1) on the basis of chemical and spectroscopic evidence. The related enone (5) undergoes a ring B aromatisation to give the phenol 7,13,15-trihydroxy-20-norlabda-5(10),6,8-triene-16,19-dioic acid 16,15-lactone 19-methyl ester (6) on treatment with mild base. Two new minor components of the extract are leonotinin [8β,17:15,16-diepoxy-9-hydroxylabda-13(16),14-dien-19,6β-olactone] (7) and 8β,17:9,13-diepoxylabdane-16,15:19,6β-diolactone (10).

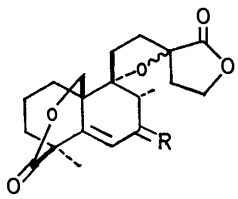
FROM an extract of Indian *L. nepetaefolia* we have isolated three new diterpenoids. The major compound was the hydroxy-dilactone, nepetaefolinol, $C_{20}H_{28}O_6$, m.p. 297°, to which we ascribe the structure (1). This contains γ- and δ-lactones and a secondary hydroxy-group [$\nu_{max.}$ ($CHCl_3$) 1779, 1716, and 3610 and 3500 cm^{-1}]. These functional groups account for five of the six oxygens. The secondary (axial) nature of the hydroxy-group follows from the formation of a monoacetate (2), m.p. 150° [δ 5·13 (m, $W_{\frac{1}{2}}$ 9 Hz)] and a ketone (3), m.p.



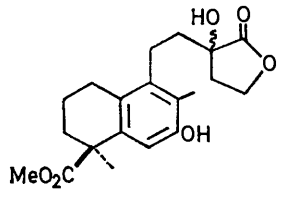
(1) R = H, β-OH
(2) R = H, β-OAc
(3) R = O

233—235° [$\nu_{max.}$ (CCl_4) 1790, 1751, and 1722 cm^{-1}], both of which lack hydroxy-absorption in the i.r. spectrum. The sixth oxygen is therefore present as an ether. The n.m.r. spectrum of nepetaefolinol reveals a tertiary (δ 1·29) and a secondary methyl group [δ 0·97 (d, *J* 7 Hz)], and five protons attached to carbon bearing an oxygen atom, two as an AB quartet [δ 4·08 and 4·66 (J_{AB} 11 Hz, 20-H_2)], and three as a complex multiplet [δ 4·3 (6-H and 15-H_2)]. In view of the previously described compounds from *L. nepetaefolia* [1,2] and related species [3-8] these data lead to the tentative structure (1) for nepetaefolinol. Proof of this was obtained in the following way.

Dehydration of nepetaefolinol (1) with thionyl chloride in pyridine afforded the olefin (4), m.p. 233—235° [δ 5·75 (q, *J* 6 and 2 Hz, H-6)], which underwent ready allylic oxidation with sodium chromate to give the enone (5), m.p. 195—197° [$\nu_{max.}$ (CCl_4) 1786, 1750, 1695, and 1636 cm^{-1}; $\lambda_{max.}$ 240 nm (ε 10,000); δ 5·98 (s, 6-H)]. A feature of the n.m.r. spectrum of the enone is a clean quartet (1 : 3 : 3 : 1, 8-H) at δ 2·89. Irradiation at this frequency caused collapse of the secondary methyl doublet while, in the reverse experiment, the δ 2·89 resonance became a sharp singlet. This clearly demonstrates the fully substituted nature of C-9. Mild base treatment of the enone (5) in methanol yielded the phenol (6), m.p. 185—186° [δ 1·52 (>CMe), 2·18 (ArMe), 3·64 (CO_2Me), 5·48 (s, phenolic OH), and 6·55 (s, aromatic proton, 6-H)], and the corresponding carboxylic acid. The typical AB quartet for the C-20 protons is absent in the spectrum of (6) but the signal for the C-15 protons appears as a complex multiplet at δ 4·38 and collapses to an AB quartet on irradiation of a two-proton triplet (14-H_2) at δ 2·46. These results confirm the formulation of the γ-lactone as



(4) R = H,H
(5) R = O



(6)

in (1) rather than the more common situation with the lactonic carbonyl group at C-15. Irradiation at δ 4·38 (15-H_2) reduces the 14-H_2 resonance to a sharp singlet

[1] J. D. White and P. S. Manchand, *J. Amer. Chem. Soc.*, 1970, **92**, 5527.
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[3] J. D. White, P. S. Manchand, and W. B. Whalley, *Chem. Comm.*, 1969, 1315.
[4] E. R. Kaplan and D. E. A. Rivett, *J. Chem. Soc.* (*C*), 1968, 262.
[5] E. R. Kaplan, K. Naidu, and D. E. A. Rivett, *J. Chem. Soc.* (*C*), 1970, 1656.
[6] G. A. Eagle and D. E. A. Rivett, *J.C.S. Perkin I*, 1973, 1701.
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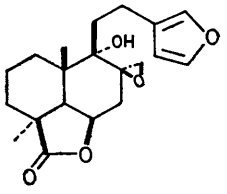
indicating that the ether terminus is at C-13 as expected. The formation of the phenol (6) involves both a β-elimination of the C-9 ether oxygen and a vinylogous retro-aldol loss of C-20 as formaldehyde. The latter was detected in the reaction mixture as its 2,4-dinitrophenylhydrazone. Acetylation of the phenol (6) under normal conditions afforded the corresponding diacetate, m.p. 236—238°.

The $Eu(fod)_3$ induced shifts * for nepetaefolinol support the relative configuration as in (1). The tertiary methyl group and one of the C-20 methylene protons move downfield markedly on successive additions of the shift reagent. The other C-20 proton and H-6 show slightly smaller shifts whereas the secondary methyl group is much less affected, in keeping with its equatorial (α) configuration. Precedent [1,2] suggests the absolute configuration shown in (1) and the positive Cotton effect ($\Delta\varepsilon$ + 1·25) of the c.d. curve of the ketone (3) confirms this.

Leonotinin, $C_{20}H_{26}O_5$, m.p. 184—186°, the second new diterpenoid from the extract, is assigned the structure (7). It has a γ-lactone and a tertiary hydroxy-group [$\nu_{max.}$ (CCl_4) 1780, 3612, and 3575 cm^{-1}] and its n.m.r. spectrum indicates the presence of two tertiary methyl groups (δ 1·10 and 1·30), an epoxide [δ 2·42 and 2·92 (ABq, J 4·5 Hz, 17-H_2)], the lactone terminus at C-6 [δ 4·84 (m, 6-H)], and a β-substituted furan (δ 6·23, 7·19, and 7·31). Confirmation of the structure and stereochemistry



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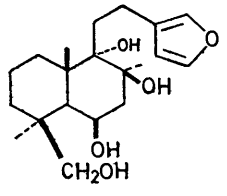

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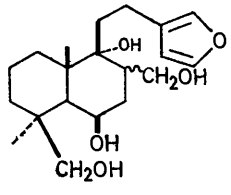

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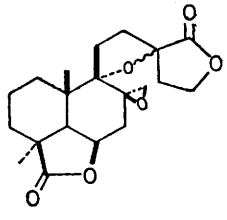

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of leonotinin was readily obtained by lithium aluminium hydride reduction which yielded two compounds. The less polar is identical with 8β-hydroxymarrubinol (8), the reduction product of 8β-hydroxymarrubiin.[3,5] The more polar compound is the isomeric tetraol (9) which presumably arises by intramolecular participation of the 9α-hydroxy-group in the initial opening of the 8,17-epoxide.

The structure of the third new compound, the dilactone (10), $C_{20}H_{26}O_6$, m.p. 221—223° [$\nu_{max.}$ (KBr) 1770 and 1780 cm^{-1}, no OH; m/e 362] is based entirely on its spectroscopic properties. The n.m.r. spectrum has signals for two tertiary methyl groups (δ 1·08 and 1·28), an 8,17-epoxide [δ 2·45 and 2·77 (ABq, J 4·5 Hz, 17-H_2)], and the protons at C-6 [δ 4·85 (1H, m)] and C-15 [δ 4·32 (2H, m)]. The complexity of the 15-H_2 multiplet clearly indicates that the orientation of the lactone ring is the same as in nepetaefolinol (1).

* Relative induced shifts (p.p.m.) for nepetaefolinol in the presence of *ca.* 0·5 mol. equiv. $Eu(fod)_3$: 3·84, tertiary Me; 3·56, 19-H; 2·76, 6-H; 2·58, 19-H; 0·72, secondary Me; 0·7, 15-H_2.

EXPERIMENTAL

I.r. spectra were run on a Perkin-Elmer grating spectrometer model 237 and u.v. spectra on a Beckmann D.U. 235; n.m.r. spectra were recorded on a Varian HA-100 spectrometer; specific rotations were measured in chloroform solution on a Zeiss polarimeter.

Isolation.—Shade-dried and coarsely powdered *L. nepetaefolia* † (whole plant; 8·5 kg) was successively extracted overnight with hexane and chloroform in the cold. Removal of the chloroform afforded a gum which was digested with ether. The residue was crystallised from chloroform–ether to yield *nepetaefolinol* (*9,13-epoxy-6β-hydroxy-8α-labdane-16,15:19,20-diolactone*) (1), m.p. 297° (decomp.) (4 g), $[\alpha]_D$ +25° (*c* 1·6), *m/e* 364 and 182 (Found: C, 65·65; H, 7·75. $C_{20}H_{28}O_6$ requires C, 65·95; H, 7·7%). The hexane extract was chromatographed over silica gel, eluting with hexane, benzene, and then chloroform. The chloroform eluate afforded crystals on addition of ether, and these gave *leonotinin* [*8β,17:15,16-diepoxy-9-hydroxylabda-13(16),14-dien-19,6β-olactone*] (7), m.p. 184—186° (from chloroform–ether), $[\alpha]_D$ +60° (*c* 2·0) (Found: C, 69·2; H, 7·8. $C_{20}H_{26}O_5$ requires C, 69·35; H, 7·5%). From the mother liquors *8β,17:9,13-diepoxylabdane-16,15:19,6β-diolactone* (10) (70 mg) was isolated by chromatography and crystallisation from ethanol, m.p. 221—223° (Found: C, 66·15; H, 7·15. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·25%).

The Acetate (2).—Nepetaefolinol (100 mg) was dissolved in pyridine (1 ml) and acetic anhydride (1·5 ml) and heated on a water-bath for 4 h. The product was filtered through a short column of silica gel in chloroform and crystallised from benzene–hexane to give *nepetaefolinol acetate* (2), m.p. 150°, $[\alpha]_D$ +35° (*c* 1·0), $\nu_{max.}$ (CCl_4) 1787 and 1743 cm^{-1}; δ 0·90 (CHMe), 1·09 (CMe), 1·98 (AcO), 4·16 and 4·56 (ABq, J 11 Hz, 20-H_2), 4·3 (m, 15-H_2), and 5·13 (m, 6-H) (Found: C, 65·35; H, 7·8. $C_{22}H_{30}O_7$ requires C, 65·05; H, 7·4%).

The Ketone (3).—Nepetaefolinol (100 mg) in acetone (10 ml) was treated with Jones reagent (1 ml) and left at room temperature for 0·5 h. Crystallisation of the product from chloroform–ether afforded the *6-ketone* (3), m.p. 233—235°, $[\alpha]_D$ −25° (*c* 2·0), δ 1·11 (CHMe), 1·33 (CMe), 2·92 (s, 5-H), and 4·3 (m, 15- and 20-H_2) (Found: C, 66·5; H, 7·4. $C_{20}H_{26}O_6$ requires C, 66·30; H, 7·2%).

Reduction of Nepetaefolinol.—Nepetaefolinol (200 mg) was reduced with an excess of lithium aluminium hydride in tetrahydrofuran under reflux for 3 h. The crude product was crystallised from ethyl acetate to give *9,13-epoxylabdane-6β,15,16,19,20-pentol*, m.p. 168—175° (Found: C, 64·35; H, 9·9. $C_{20}H_{36}O_6$ requires C, 64·5; H, 9·7%).

The Olefin (4).—Nepetaefolinol (100 mg) in pyridine (5 ml) and thionyl chloride (0·2 ml) was kept overnight at room temperature. The solvent was removed *in vacuo* and the crude residue filtered through a short column of silica gel in methylene chloride. Crystallisation from benzene–ether afforded *9,13-epoxylabd-5-ene-16,15:19,20-diolactone* (4) (65 mg), m.p. 233—235°, $[\alpha]_D$ −80° (*c* 2·0), $\nu_{max.}$ (CCl_4) 1784 and

† The plant was collected in the flowering season (December—January) from the King Institute, Guindy area.

1738 cm^{-1}; δ 1·04 (CHMe), 1·39 (CMe), 4·4 (m, 15- and 20-H_2), and 5·75 (q, *J* 6 and 2 Hz, 6-H) (Found: C, 69·4; H, 7·7. $C_{20}H_{26}O_5$ requires C, 69·35; H, 7·5%).

The Enone (5).—The olefin (4) (165 mg) was warmed at 40° in acetic acid (2 ml) and acetic anhydride (2 ml) and dry sodium chromate (150 mg) added in portions. The mixture was stirred overnight, then the crude product was filtered through silica gel in chloroform and crystallised from benzene–ether to yield 9,13-*epoxy*-7-*oxolabd*-5-*ene*-16,15: 19,20-*diolactone* (5) (120 mg), m.p. 195—197°, $[\alpha]_D$ −95° (*c* 2·0), δ 1·16 (CHMe), 1·40 (CMe), 2·89 (q, *J* 6 Hz, 8-H), 4·3 (m, 15- and 20-H_2), and 5·98 (s, 6-H) (Found: C, 66·8; H, 6·7. $C_{20}H_{24}O_6$ requires C, 66·7; H, 6·65%).

The Phenol (6).—The enone (5) (250 mg) in methanol (5 ml) was treated with sodium carbonate (250 mg) dissolved in a minimum amount of water, and the solution was refluxed for 0·5 h. The mixture was cooled, acidified with dilute hydrochloric acid, and extracted with ethyl acetate. The ethyl acetate layer was washed several times with aqueous sodium hydrogen carbonate solution and then evaporated. The neutral product was crystallised from ether to give 7,13,15-*trihydroxy*-20-*norlabda*-5(10),6,8-*triene*-16,19-*dioic acid* 16,15-*lactone* 19-*methyl ester* (6) (80 mg), m.p. 185—186°, $[\alpha]_D$ −70° (*c* 1·7), $\nu_{max.}$ ($CHCl_3$) 3600, 3570sh, 3445br, 1776, 1723, and 1603 cm^{-1}; δ 1·52 (CMe), 2·18 (ArMe), 2·46 (t, 14-H_2), 2·93 (OH), 3·64 (CO_2Me), 4·38 (m, 15-H_2), 5·48 (s, phenolic OH), and 6·55 (s, 6-H). The phenol gave a blue colour with iron(III) chloride and ferricyanide (Found: C, 66·6; H, 7·5. $C_{20}H_{26}O_6$ requires C, 66·30; H, 7·2%).

The hydrogencarbonate layer was acidified and extracted with ethyl acetate to yield an acidic fraction (160 mg). Methylation with diazomethane and crystallisation from ether afforded the phenol (6), m.p. 185—186°, identical with the compound obtained from the neutral fraction.

An excess of 2,4-dinitrophenylhydrazine reagent was added to the initial aqueous layer above and the mixture refluxed for 10 min, and cooled. The yellow crystalline solid which precipitated gave needles, m.p. 164—165° (from methanol) (65 mg), identical with formaldehyde 2,4-dinitrophenylhydrazone.

Acetylation of the phenol (6) under normal conditions and crystallisation from ether–hexane yielded the corresponding *diacetate*, m.p. 236—238°, $[\alpha]_D$ −38° (*c* 1·70), $\nu_{max.}$ (CCl_4) 1792, 1752, and 1735 cm^{-1}; δ 1·58 (CMe), 2·14 (ArMe), 2·20 and 2·34 (AcO), 3·67 (CO_2Me), 4·4 (m, 15-H_2), and 6·72 (c, 6-H) (Found: C, 64·3; H, 6·85. $C_{24}H_{30}O_8$ requires C, 64·6; H, 6·75%).

Reduction of Leonotinin (7).—Leonotinin (250 mg) in tetrahydrofuran was treated with lithium aluminium hydride (250 mg) and the mixture refluxed for 2 h. The crude product consisted of two compounds which were separated by chromatography over silica gel in chloroform. The less polar product (100 mg) was recrystallised from ethyl acetate–petroleum, m.p. 151—153°, $[\alpha]_D$ +35° (*c* 0·7) (Found: C, 68·4; H, 9·3. Calc. for $C_{20}H_{32}O_5$: C, 68·1, H, 9·1%), and was identical with 8β-hydroxymarrubinol (8).

The more polar product was the isomeric 15,16-*epoxy-labda*-13(16),14-*diene*-6β,9,17,19-*tetrol* (9) (80 mg), m.p. 199—200° (from ethyl acetate–hexane), $[\alpha]_D$ +35° (*c* 1·0 in EtOH) (Found: C, 68·1; H, 9·15. $C_{20}H_{32}O_5$ requires C, 68·15; H, 9·15%).

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