# Diterpenoids of *Leonotis* Species. Part 5.[1] Leonitin, a 9,13-Epoxylabdane from *L. leonitis* R. Br.

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The grindelane diterpenoid, leonitin, $C_{22}H_{30}O_7$, isolated from *L. leonitis* R. Br., is identified as 20-acetoxy-9,13-epoxylabda-6(4),16(15)-diol dilactone (1), and has been related to nepetaefolinol from *L. nepetaefolia*.

LEONITIN, $C_{22}H_{30}O_7$, which has been isolated from the leaves of *L. leonitis*, contains a γ-lactone and an ester group, gives a negative Ehrlich test, and absorbs no hydrogen on catalytic hydrogenation. The n.m.r. spectra of leonitin and compound (2), from *L. leonurus* [2] are very similar (see Experimental section), the only difference being that the C-20 tertiary methyl singlet at δ 1.10 in compound (2) is replaced by an acetate methyl singlet at δ 2.02 in leonitin, and the C-16 methylene AB quartet (*J* 9 Hz) centred at δ 4.19 in compound (2) is replaced by a complicated 4-proton signal between δ 4.05 and 4.32, probably due to two AB quartets. These differences suggest that leonitin is the 20-acetoxy derivative of (2).

The mass spectrum of leonitin contains a large molecular ion (14%) and a base peak at *m/e* 181, shown by accurate mass measurements to be due to the fragment (3a). It is known that a large molecular ion and,

(1) R = OAc
(2) R = H

(3)
a; $R^1,R^2$ = $CH_2{\cdot}CO{\cdot}O{\cdot}CH_2$
b; $R^1$ = $CH_2{\cdot}CH_2OH$; $R^2$ = $CH_2OH$
c; $R^1,R^2$ = $CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2$
d; $R^1$ = Et, $R^2$ = Me

(4)
a; $R^1,R^2$ = $CH_2{\cdot}CO{\cdot}O{\cdot}CH_2$
b; $R^1$ = $CH_2{\cdot}CH_2OH$; $R^2$ = $CH_2OH$
c; $R^1,R^2$ = $CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2$
d; $R^1,R^2$ = $CH_2{\cdot}CH_2{\cdot}O{\cdot}CO$

(5)
a; $R^1$ = $CH_2{\cdot}CH_2OH$; $R^2$ = $CH_2OH$
b; $R^1,R^2$ = $CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2$

(6)

(7)
a; $R^1,R^2$ = $CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2$
b; $R^1$ = Et, $R^2$ = Me

[1] Part 4, G. A. Eagle and D. E. A. Rivett, *J.C.S. Perkin I*, 1973, 1701.

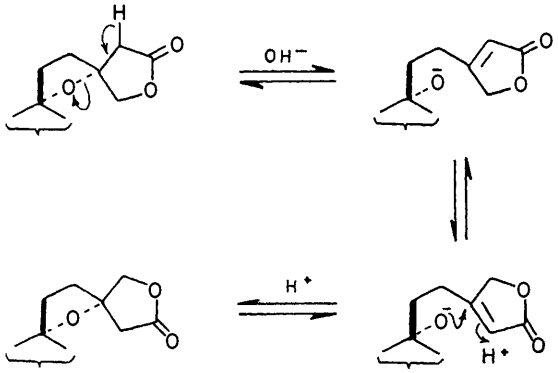
[2] E. R. Kaplan and D. E. A. Rivett, *J. Chem. Soc.* (*C*), 1968, 262.

particularly, a base peak near the centre of the spectrum are typical of 9,13-epoxylabdanes.[3]

On hydrolysis leonitin consumes 3.0 mol. equivalents of alkali to give acetic acid and an alcohol, $C_{20}H_{28}O_6$, containing both a γ- and a δ-lactone group. This product (4a) does not regenerate leonitin on acetylation and accordingly ring closure must have occurred between the primary hydroxy-group at C-20 and the carboxy-group at C-19 to afford the less strained six-membered ring. Several unsuccessful attempts were made to hydrolyse the acetate group in leonitin without affecting the lactone rings in order to effect an interconversion between leonitin and compound (2).

In some samples of saponified leonitin and its acetate the C-14, C-16, and C-20 methylene signals as well as the C-17 methyl doublet clearly exhibited two sets of signals, approximately equal in size and separated by 3 Hz. Products obtained under identical experimental conditions varied, some showing splitting and others not; this behaviour is best explained by the presence of two C-13 epimers formed from the readily opened upper lactone ring (see Scheme 1).

Sodium borohydride reduced only the γ-lactone ring in saponified leonitin to afford the triol (4b). This triol was converted into the 15,16-epoxide (4c) with either tosyl chloride in pyridine or by dilute acid; both methods were used to convert lagochilin (5a) into



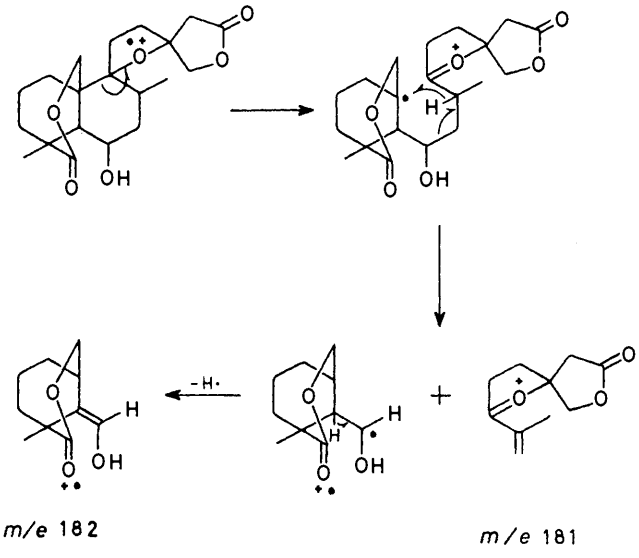
SCHEME 1

anhydrolagochilin (5b).[4] As expected, the mass spectra of the triol (4b) and the epoxide (4c) show base peaks at *m/e* 185 and 167 due to the ions (3b) and (3c) respectively.

Saponified leonitin (4a) is clearly closely related to nepetaefolinol (4d), present in Indian *L. nepetaefolia*.[5] Leonitin was reduced by lithium aluminium hydride to a pentaol (6), shown to be identical with the product obtained by similar reduction of a sample of nepetaefolinol.

The structure originally proposed for compound (2) has since been proven by correlation of this compound with marrubiin [6] whose stereochemistry is firmly established.[7-9] However, the stereochemistry at the C-13 spiro-centre in compound (2), leonitin, and nepetaefolinol, remains undetermined although the above chemical interrelation between leonitin and nepetaefolinol indicates that in all three compounds it must be the same.*

Like nepetaefolinol the base peak in the spectrum of saponified leonitin occurs at *m/e* 182 instead of the expected *m/e* 181. This deviation is explained by the




SCHEME 2

following fragmentation pattern (Scheme 2) which is supported by accurate mass measurements for both these compounds.

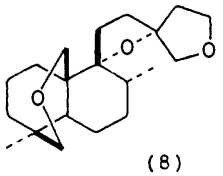
When compound (2) was reduced with lithium aluminium hydride and the product tosylated and reduced, the triepoxide (7a), $C_{20}H_{22}O_3$, which showed one secondary and two tertiary methyl groups in its n.m.r. spectrum, was produced. In an effort to relate leonitin chemically with compound (2) we tried to convert the pentaol (6) into the triepoxide (7a) by tosylation followed by reduction with lithium aluminium hydride. Two products were obtained, the diepoxide (7b), and the triepoxide (8); t.l.c. showed that none of the desired ether was formed. The n.m.r. spectrum of the major product (7b) indicated the presence of one primary, one secondary, and three tertiary methyl groups, while the spectrum of the minor product (8) contained only one secondary and one tertiary methyl group.

The mass spectra of these three epoxides were particularly informative. The ether (7a) showed two main peaks due to the fragments (3c) (38%) and (9) (100%),

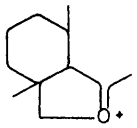
* For a review of naturally occurring 9,10-epoxylabdanes see ref. 3.

[3] D. E. A. Rivett, *ChemSA*, 1976, 7.
[4] D. E. A. Rivett, *J. S. African Chem. Inst.*, 1975, 305.
[5] K. K. Purushothaman, S. Vasanath, and J. D. Connolly, *J.C.S. Perkin I*, 1975, 2661.
[6] J. W. B. Fulke, M. S. Henderson, and R. McCrindle, *J. Chem. Soc. (C)*, 1968, 807.
[7] R. A. Appleton, J. W. B. Fulke, M. S. Henderson, and R. McCrindle, *J. Chem. Soc. (C)*, 1967, 1943.
[8] L. J. Stephens and D. M. S. Wheeler, *Tetrahedron*, 1970, **26**, 1561.
[9] L. Mangoni and M. Adinolfi, *Tetrahedron Letters*, 1968, 269; *Gazz. Chim. Ital.*, 1968, **98**, 122.

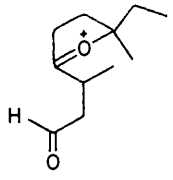
and the ether (7b) peaks due to the fragments (3d) (100%), (9) (100%), and (10) (44%). The ether (8) had peaks due to the fragments (3c) (30%) and (11) (30%) with the base peak ($C_{11}H_{17}O_3$) at *m/e* 197 presumably arising from extensive rearrangement. All the



(8)



(9)



(10)

(11)

proposed fragments are supported by accurate mass measurements.

## EXPERIMENTAL

For general experimental details see ref. 1. Unless stated otherwise all i.r. spectra and specific rotations were determined in chloroform. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. Mass spectra were taken on an A.E.I. MS-9 instrument at inlet temperatures of 120—150 °C, and n.m.r. spectra at 100 MHz on a Varian HA 100 spectrometer.

*Isolation.*—The acetone extract of the dried leaves (5.4 kg) of *L. leonitis* (Rhodes University Herbarium No. 21877), collected during December 1970, was shaken with activated charcoal and evaporated. The resulting gum was dissolved in chloroform and washed with water. Evaporation and crystallisation from ethyl acetate afforded crude leonitin (29.7 g, 0.47%) showing a single spot on t.l.c. Leonitin, purified by crystallisation from ethanol and sublimation at 220 °C and 0.5 mmHg, had m.p. 242—243 °C, $[\alpha]_D$ −1° (*c* 1.2); $\nu_{max.}$ 1 770 and 1 740 $cm^{-1}$; $\delta(CDCl_3)$ 4.68 (1 H, t, *J* 5 Hz, 6-H), 4.05—4.32 (4 H, m, 16-$H_2$ and 20-$H_2$), 2.75 (2 H, q, *J* 17 Hz, 14-$H_2$), 2.02 (3 H, s, OAc), 1.27 (3 H, s, 18-$H_3$), and 0.88 (3 H, d, *J* 6 Hz, 17-$H_3$); *m/e* 408 ($M^+$, 14%), 347 (5), 247 (16), 183 (29), 182 (13), and 181 (100) (Found: C, 65.4; H, 7.4. $C_{22}H_{30}O_7$ requires C, 65.0; H, 7.4%).

Compound (2) showed $\delta(CDCl_3)$ 4.68 (1 H, t, *J* 5 Hz, 6-H), 4.19 (2 H, q, *J* 9 Hz, 16-$H_2$), 2.73 (2 H, q, *J* 17 Hz, 14-$H_2$), 1.28 (3 H, s, 18-$H_3$), 1.10 (3 H, s, 20-$H_3$), and 0.84 (3 H, d, *J* 6.5 Hz, 17-$H_3$).

*Saponification of Leonitin.*—Leonitin (3.0 g) was refluxed in ethanolic 0.5M-potassium hydroxide for 1 h, after which water was added to the mixture and ethanol removed under reduced pressure. The hot solution was acidified with hydrochloric acid, and the saponified leonitin extracted continuously with ether and crystallised from benzene (1.7 g), m.p. 238 °C, $[\alpha]_D$ +30° (*c* 1.3 in ethanol); $\nu_{max.}$ 3 470, 1 785, and 1 710 $cm^{-1}$; $\delta(CDCl_3)$ 4.65 (1 H, d, *J* 11 Hz, 6-H), 3.95—4.35 (4 H, m, 16-$H_2$ and 20-$H_2$), 2.72 (2 H, q, *J* 17 Hz, 14-$H_2$), 1.26 (3 H, s, 18-$H_3$), and 0.88 (3 H, d. *J* 6 Hz, 17-$H_3$); *m/e* 364 ($M^+$, 6%), 346 (26), 212 (13), 211 (91), 193 (12), 183 (43), 182 (100), and 181 (91) (Found: C, 65.8; H, 8.0. $C_{20}H_{28}O_6$ requires C, 65.9; H, 7.7%).

Nepetaefolinol had *m/e* 364 ($M^+$, 2%), 346 (19), 212 (7), 211 (54), 193 (11), 183 (21), 182 (100), and 181 (34).

The aqueous solution after ether extraction was steam-distilled and acetic acid identified in the distillate as the *p*-bromophenacyl ester, m.p. and mixed m.p. 84 °C.

*Acetylation of Saponified Leonitin.*—Saponified leonitin (100 mg) was refluxed in acetic anhydride (3 ml) and pyridine (0.2 ml) for 4 h to give the *acetate* (50 mg), m.p. 295—296 °C (needles from chloroform–hexane), $[\alpha]_D$ +12° (*c* 0.8); $\nu_{max.}$ 1 790, 1 730, and 1 740sh $cm^{-1}$; $\delta(CDCl_3)$ 5.14 (1 H, q, *J* 3 Hz, 6-H), 4.6—4.0 (4 H, m, 16-$H_2$ and 20-$H_2$), 2.72 (2 H, q, *J* 17 Hz, 14-$H_2$), 2.0 (3 H, s, OAc), 1.12 (3 H, s, 18-$H_3$), and 0.86 (3 H, d, *J* 6 Hz, 17-$H_3$); *m/e* 406 ($M^+$, trace), 347 (23), 346 (100), 194 (23), 182 (4), and 181 (19) (Found: C, 65.0; H, 7.4. $C_{22}H_{30}O_7$ requires C, 65.0; H, 7.4%).

Nepetaefolinol acetate had *m/e* 406 ($M^+$, 1%), 347 (45), 346 (100), 194 (25), 182 (5), and 181 (26).

*Reduction of Saponified Leonitin with Sodium Borohydride.*—A mixture of saponified leonitin (160 mg), sodium borohydride (0.20 g), and ethanol (25 ml) was stirred overnight. Water was added and the ethanol removed under reduced pressure. The hot solution was acidified with hydrochloric acid and the product was extracted continuously with ether. The ether was evaporated and boric acid in the residue was removed by evaporation with methanol. Crystallisation from methanol afforded the crude *triol* (4b) (85 mg), m.p. 235—245 °C, raised on recrystallisation to m.p. 262—264 °C, $[\alpha]_D$ +29° (*c* 1.2); $\nu_{max.}$(KBr) 3 360 and 1 700 $cm^{-1}$; *m/e* 368 ($M^+$, trace), 322 (12), 321 (56), 291 (43), 215 (23), 186 (26), 185 (100), 175 (21), 173 (16), 172 (70), 167 (22), 163 (9), and 161 (11) (Found: C, 65.25; H, 8.8. $C_{20}H_{32}O_6$ requires C, 65.2; H, 8.75%).

*Dehydration of the Triol* (4b) *to the Ether* (4c).—(*a*) A solution of the triol (100 mg) and toluene-*p*-sulphonyl chloride (150 mg) in dry pyridine (6 ml) was refluxed for 24 h. Ice was added, the solution was extracted with ether and the extract was washed with dilute hydrochloric acid and water and then evaporated. The residue was triturated with benzene and crystallised from methanol to afford the *ether* (4c) as prisms (30 mg), m.p. 305—308 °C, $[\alpha]_D$ +30° (*c* 0.4 in ethanol); $\nu_{max.}$(KBr) 3 470 and 1 700 $cm^{-1}$; *m/e* 350 ($M^+$, 3), 197 (11), 168 (40), and 167 (100) (Found: C, 68.50; H, 8.5. $C_{20}H_{30}O_5$ requires C, 68.55; H, 8.6%).

(*b*) A mixture of the triol (30 mg), ethanol (2 ml), water (1.6 ml), and concentrated hydrochloric acid (0.4 ml) was refluxed overnight. The resulting precipitate (19 mg), m.p. 300—307 °C, was identical (mixed m.p. and i.r. spectrum) with material from experiment (*a*).

*Reduction of Leonitin with Lithium Aluminium Hydride.*—Leonitin (90 mg) was refluxed with lithium aluminium hydride (0.15 g) in tetrahydrofuran for 2 h to afford the *pentaol* (6) as needles (70 mg) (from ethyl acetate), m.p. 169 °C, $[\alpha]_D$ −16° (*c* 1.0 in ethanol); *m/e* 340 (16), 322 (13), 215 (56), 197 (64), 187 (19), 186 (100), 185 (58), 180 (12), 179 (18), 175 (19), 173 (19), 172 (54), 169 (25), 168 (22), 167 (41), 163 (25), and 161 (16) (Found: C, 64.8; H, 9.9. $C_{20}H_{36}O_6$ requires C, 64.5; H, 9.7%).

Similar reduction of nepetaefolinol gave a product identical (mixed m.p., t.l.c., i.r., and rotation) with that from leonitin.

*Conversion of Compound* (2) *into the Triepoxide* (7a).—A solution of the lithium aluminium hydride reduction product of compound (2) [2] (300 mg) and toluene-*p*-sulphonyl chloride (1.6 g) in dry pyridine (10 ml) was left at 0 °C for 4 days. Ice was added to the mixture which was then extracted with ether; the extract was washed with dilute hydrochloric acid and water, dried, and evaporated. The resulting gum was distilled at 120 °C and 0.1 mmHg and recrystallised from hexane to afford needles (35 mg) of the *triepoxide* (7a), m.p. 115 °C, $[\alpha]_D$ −12° (*c* 1.5); δ($CDCl_3$) 4.10 (1 H, t, *J* 5 Hz, 6-H), 3.86 (2 H, d of t, *J* 7 and 2 Hz, 15-$H_2$), 3.61 (2 H, ABq, *J* 8 Hz, 19-$H_2$), 1.16 (3 H, s, 18-$H_3$), 1.10 (3 H, s, 20-$H_3$), and 0.86 (3 H, d, *J* 6 Hz, 17-$H_3$); *m/e* 320 ($M^+$, 20), 167 (38), 154 (34), and 153 (100) (Found: C, 75.2; H, 10.0. $C_{20}H_{32}O_3$ requires C, 75.0; H, 10.1%).

*Conversion of Leonitin into the Diepoxide* (7b) *and the Triepoxide* (8).—A solution of the pentaol (6) (1.8 g) and toluene-*p*-sulphonyl chloride (9.0 g) in dry pyridine (35 ml) was left at −30 °C for 4 days and worked up as in the previous experiment. The crude product was refluxed overnight with lithium aluminium hydride (3.0 g) in tetrahydrofuran (150 ml) to afford an oil (1.8 g). A sample (1.4 g) was chromatographed on alumina, increasing concentrations of ethyl acetate in hexane being used as eluant. The two major fractions were further purified by low pressure chromatography on Woelm silica gel (0.03—0.06 mm) in ethyl acetate–hexane (1 : 10) and (1 : 5) to afford fractions which on crystallisation from hexane at −20 °C gave (i) the *diepoxide* (7b) as prisms (0.14 g from 0.45 g fraction), m.p. 62—63 °C, $[\alpha]_D$ −1° (*c* 0.9); δ($CDCl_3$) 4.08 (1 H, d, *J* 5 Hz, 6-H), 3.57 (2 H, ABq, *J* 9 Hz, 19-$H_2$), 1.29 (6 H, s, 18-$H_3$ and 20-$H_3$), 0.91 (3 H, d, *J* 6 Hz, 17-$H_3$), 0.90 (3 H, s, 16-$H_3$), and 0.86 (3 H, t, *J* 6 Hz, 15-$H_3$); *m/e* 306 ($M^+$, 15%), 183 (44), 154 (31), and 153 (100) (Found: C, 78.5; H, 11.2. $C_{20}H_{34}O_2$ requires C, 78.4; H, 11.2%); and (ii) the *triepoxide* (8) as prisms (70 mg from 290 mg), m.p. 83—84 °C, $[\alpha]_D$ −12° (*c* 1.2); δ($CDCl_3$) 0.90 (3 H, s, 18-$H_3$), 0.92 (3 H, d, *J* 6 Hz, 17-$H_3$); *m/e* 320 ($M^+$, 20%), 197 (100), 167 (30), 153 (30) (Found: C, 75.1; H, 10.2. $C_{20}H_{32}O_3$ requires C, 75.0; H, 10.1%).

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