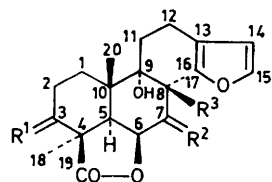


Structure of Ballotinone, a Diterpenoid from *Ballota nigra*

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Ballotinone has been shown on the basis of its ^{13}C n.m.r. spectrum to be 7-oxomarrubiin [15,16-epoxy-9-hydroxy-7-oxo-8 β H-labda-13(16),14-dien-19,6 β -olactone].

BLACK HOREHOUND [*Ballota nigra* (Labiatae)] is a Mediterranean medicinal herb which has been used to adulterate white horehound (*Marrubium vulgare*).¹ Many bi- and tri-cyclic diterpenoids have been isolated from the Labiatae and we are now studying the diterpenoid content of some *Ballota* species. Marrubiin (I) has been isolated² from *B. foetida* (= *B. nigra* sub. sp. *foetida*).³ In the present work, extraction of *B. nigra* with ethyl acetate afforded some new diterpenoids. We now show that one of these, ballotinone, $\text{C}_{20}\text{H}_{26}\text{O}_5$, is 7-oxomarrubiin (4). T.l.c. of the extract also indicated the presence of traces of marrubiin (I).



- (1) $\text{R}^1 = \text{R}^2 = \text{H}_2, \text{R}^3 = \text{H}$
 (2) $\text{R}^1 = \text{O}, \text{R}^2 = \text{H}_2, \text{R}^3 = \text{H}$,
 (3) $\text{R}^1 = \text{R}^2 = \text{H}_2, \text{R}^3 = \text{OH}$,
 (4) $\text{R}^1 = \text{H}_2, \text{R}^2 = \text{O}, \text{R}^3 = \text{H}$,
 (5) $\text{R}^1 = \text{H}_2, \text{R}^2 = \alpha\text{-H}, \beta\text{-OH}, \text{R}^3 = \text{H}$

Ballotinone (4) had i.r. absorption at 3 550 (OH), 1 760 (γ -lactone), 1 700 (sat. C=O), and 875 cm^{-1} (furan), thus accounting for the five oxygen atoms. It gave a positive Ehrlich test for the presence of a furan ring. The ^1H n.m.r. spectrum showed resonances attributable to two tertiary methyl groups (δ 0.79 and 1.30) and a secondary methyl group (δ 1.25, J 8 Hz). The latter was coupled to a single proton quartet (δ 2.83); irradiation at this frequency led to collapse of the methyl doublet to a singlet.

¹ 'A Dictionary of Plants Used by Man,' ed. G. Usher, Constable, London, 1974.

A single proton doublet (δ 4.91, J 7 Hz) was assigned to the CH \cdot O terminus of the γ -lactone ring. Irradiation at this frequency led to collapse of a methine proton doublet (δ 3.05, J 7 Hz) to a singlet. In addition there were three single proton resonances (δ 6.27, 7.25, and 7.36) attributable to a β -substituted furan ring. The noise-decoupled and single-frequency off-resonance decoupled ^{13}C n.m.r. spectra of ballotinone confirmed the presence of three methyl groups and showed that the other carbon atoms were disposed as five methylene, six methine (three in the furan ring), and six quaternary carbon atoms (including one in the furan ring, one in a tertiary alcohol, and two in carbonyl groups).

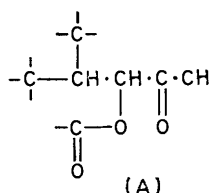
The relationship of the saturated ketone group to the γ -lactone ring was established by reduction of ballotinone with sodium borohydride to give an alcohol (5) (ν_{max} 3 590, 3 410, 1 775, and 875 cm^{-1}). The lactonic CH \cdot O proton resonance (δ 4.84) in the product (5) appeared as a quartet (J 4 and 6 Hz). Irradiation at this frequency led to collapse of a single proton quartet (δ 3.68) to a doublet (J 11 Hz). This quartet was associated with the new secondary alcohol. A methine doublet (δ 2.30, J 4 Hz) also collapsed to a singlet. Thus ballotinone contains the system (A).

The formula of ballotinone embraces eight double bond equivalents of which six are included in the lactone ring,

² J. Balansard, *Compt. rend. Soc. Biol.*, 1934, **117**, 1014 (*Chem. Abs.*, 1935, **29**, 1578).

³ 'Flora Europaea,' ed. T. G. Tutin *et al.*, Cambridge, 1972, vol. 3, p. 150.

the furan ring, and the ketone. The compound was thus considered to be a bicyclic diterpenoid, possibly related to marrubiin (1), with which it co-occurred. The structure followed from a comparison of the ^{13}C n.m.r. spectra of marrubiin (1),⁴ peregrinone (2),⁵ and leonotin (3)⁶ with



those of ballotinone (4) and the alcohol (5). The resonances of the known diterpenoids were assigned on the following basis. The three methyl resonances of C-17, -18, and -20 were distinguished from each other by the shielding effect of the C-3 oxo-group of peregrinone on

presence of a C-3 ketone (*cf.* gibberellin A₉ and A₄ ketone, $\Delta\delta$ 1.3 p.p.m.).⁷ The β -furan resonance at 110.7 p.p.m. was clearly separated from the two α -carbon resonances at 138.5 and 142.9 p.p.m. which were distinguished by the known effect of an alkyl group on olefinic carbon resonances. The singlet resonances were associated with C-4, -9, -10, and -13. The signals assigned to C-9 and -13 were in the anticipated ranges, and C-4 and -10 were distinguished by the deshielding effect (9.4 p.p.m.) of the adjacent C-3 carbonyl group on C-4 in peregrinone. After this work was completed the ^{13}C n.m.r. spectra of some bicyclic diterpenoids were assigned.⁸ These included marrubiin, and our assignments are in accord.

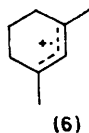
It was then possible to locate the carbonyl group of ballotinone on the basis of these assignments. In particular the ^{13}C n.m.r. signal associated with C-7 was displaced downfield to 109.0 p.p.m. and the doublet associated with C-8 showed deshielding by 19.1 p.p.m. On the

Carbon-13 n.m.r. signals (p.p.m. from Me₄Si)

| Compd. | Carbon atom | | | | | | | | | | | | | | | | | | | |
|--------|-------------|------|-------------------|------|------|------|-------|------|------|------|-------------------|------|-------|-------|-------|-------|------|-------------------|-------|-------------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| (1) | 35.1 | 18.1 | 28.5 ^a | 43.8 | 44.8 | 76.3 | 31.4 | 32.3 | 75.6 | 39.7 | 28.3 ^a | 21.0 | 125.1 | 110.7 | 142.9 | 138.5 | 16.6 | 22.9 ^b | 184.0 | 22.3 ^b |
| (2) | 34.6 | 34.1 | 207.1 | 53.2 | 46.5 | 75.1 | 31.0 | 31.5 | 75.1 | 40.0 | 28.7 | 21.0 | 124.8 | 110.6 | 143.1 | 138.5 | 16.0 | 18.1 | 174.8 | 20.4 |
| (3) | 32.1 | 17.9 | 28.8 | 42.3 | 43.9 | 74.4 | 42.3 | 79.7 | 75.6 | 40.1 | 31.4 | 20.7 | 125.4 | 110.8 | 143.0 | 138.6 | 31.0 | 21.0 | 182.5 | 25.5 |
| (4) | 37.9 | 18.0 | 29.0 | 41.6 | 44.7 | 75.5 | 209.0 | 51.4 | 77.9 | 40.7 | 29.0 | 18.2 | 124.2 | 110.6 | 143.2 | 138.8 | 15.8 | 26.4 ^a | 180.1 | 18.0 ^a |
| (5) | 35.1 | 18.0 | 28.6 ^a | 44.5 | 45.4 | 77.7 | 71.8 | 41.3 | 77.7 | 39.7 | 28.4 ^a | 21.0 | 124.9 | 110.7 | 143.1 | 138.6 | 12.3 | 22.8 ^b | 183.0 | 22.3 ^b |

^{a, b} These assignments may be interchanged.

C-18. There is a comparable shielding (6.9 p.p.m.) of C-18 on introduction of the C-3 carbonyl group into the gibberellins.⁷ The 8β -hydroxy-group of leonotin produces a deshielding effect both on the adjacent C-17 and on C-20, with which it has a diaxial interaction. The methylene triplet resonances associated with C-1, -2, -3, -7, -11, and -12 were distinguished as follows. A triplet resonance at 28.3 p.p.m. in the spectrum of marrubiin, assigned to C-3, was replaced by a singlet at 207.1 p.p.m. in the 3-ketone, and another triplet at 18.1 p.p.m. which was assigned to C-2 was displaced downfield to 34.1 p.p.m. in a manner characteristic of a methylene ketone. The two triplet resonances at 35.4 and 31.4 p.p.m. in the spectrum of marrubiin, were assigned to C-1 and -7, respectively. Comparison with leonotin showed that the 35.4 p.p.m. resonance had moved upfield to 32.1 p.p.m., characteristic of a carbon atom δ to a hydroxy-group, and that at 31.4 p.p.m. was deshielded by the additional C-8 hydroxy-group. The remaining triplet resonances at 28.6 and 21.0 p.p.m. were then assigned to C-11 and -12, respectively. Amongst the doublet resonances, that



assigned to C-8 was replaced by a singlet in 8β -hydroxy-marrubiin, and C-5 showed a small downfield shift in the

⁴ R. A. Appleton, J. W. B. Fulke, M. S. Henderson, and R. McCrindle, *J. Chem. Soc. (C)*, 1967, 1943, and references cited therein.

⁵ L. Canonica, B. Rindone, C. Scholastico, G. Ferrari, and C. Casagrande, *Tetrahedron Letters*, 1968, 3149.

other hand the signals associated with ring A appeared in their anticipated regions. In the 7-alcohol the triplet methylene signal associated with C-7 in marrubiin was again missing, and the C-8 doublet was displaced downfield. Hence ballotinone was identified as 7-oxomarrubiin (4). The mass spectra of compounds (4) and (5) can be readily interpreted in these terms. They contain significant ions at m/e 81 and 95 which may be associated with furanoid fragments, and an ion (the base peak in the spectrum of ballotinone) at m/e 109 (6) associated with ring A. In addition in the spectrum of ballotinone there was an abundant ion at m/e 251 corresponding to loss of the side chain and an ion at m/e 223 arising by further decarbonylation. The C-7 stereochemistry of the alcohol (5) was defined by the magnitude of the proton coupling constants $J_{7,8}$ (11 Hz) and $J_{6,7}$ (6 Hz). These imply diaxial and equatorial-axial proton relationships and hence a 7β -equatorial alcohol.

EXPERIMENTAL

Light petroleum refers to the fraction, b.p. 60–80°. I.r. spectra were determined for Nujol mulls with a Perkin-Elmer 257 instrument; n.m.r. spectra were determined for solutions in deuteriochloroform with a Varian HA 100 (^1H) or JEOL PS 100 (^{13}C) spectrometer; optical rotations were determined for solutions in chloroform; mass spectra were determined at 70 eV with an A.E.I. MS30 instrument.

Extraction of Ballota nigra sub. sp. foetida.—Air-dried

⁶ E. R. Kaplan, K. Naidu, and D. E. A. Rivett, *J. Chem. Soc. (C)*, 1970, 1655.

⁷ R. Evans, J. R. Hanson, and M. Siverns, *J.C.S. Perkin I*, 1975, 1574.

⁸ S. Almquist, C. R. Enzell, and F. W. Wehrli, *Acta Chem. Scand.*, 1975, B29, 695.

leaves (0.6 kg) collected in the Botanic Gardens, Palermo, in June 1974, were powdered and extracted (Soxhlet) with ethyl acetate. The extract was washed with aqueous 10% sodium carbonate, 1% hydrochloric acid, and water, dried, and evaporated. The residue (15 g) was chromatographed on a dry silica gel column (Merck; deactivated with 15% water). Elution with light petroleum gave plant waxes. Elution with 30% ethyl acetate–light petroleum gave a crude crystalline fraction (0.6 g) which was rechromatographed in the same solvent system to afford ballonigrin (unidentified) (150 mg), m.p. 212°, $[\alpha]_D^{20} +2^\circ$ (c 0.25). T.l.c. of the mother liquors revealed traces of marrubiin. Further elution of the first column afforded *ballotinone* (300 mg), which crystallized from ethyl acetate–light petroleum as prisms, m.p. 194°, $[\alpha]_D^{20} +57^\circ$ (c 0.3) (Found: C, 68.9; H, 7.7. $C_{20}H_{26}O_5$ requires C, 69.4; H, 7.5%), ν_{\max} 3 550, 1 760, 1 700, and 875 cm^{-1} , δ 0.79 (3 H, s), 1.25 (3 H, d, J 8 Hz), 1.30 (3 H, s), 2.83 (1 H, q, J 8 Hz), 3.05 (1 H, d, J 7 Hz), 4.91 (1 H, d, J 7 Hz), 6.27 (1 H, m), 7.25 (1 H, m, $W_{\frac{1}{2}}$ 4 Hz), and 7.36 (1 H, m, $W_{\frac{1}{2}}$ 3 Hz), m/e 346, 289, 251, 223, 177, 123, 109 (base peak), 95, and 81. Further elution of the column with 50% ethyl acetate–light petroleum gave ballot-

enol (unidentified) (350 mg), which crystallized from ethyl acetate as needles, m.p. 140–145° (decomp.), $[\alpha]_D^{20} 0^\circ$ (c 0.38 in pyridine).

Reduction of Ballotinone.—The diterpenoid (35 mg) in methanol (5 ml) was treated with sodium borohydride (30 mg) for 2 h at room temperature. The solution was treated with a drop of acetic acid, then concentrated, and the *alcohol* (5) was recovered in ethyl acetate. It crystallized from ethyl acetate–light petroleum as needles, m.p. 169° (Found: C 68.7; H, 7.9. $C_{20}H_{28}O_5$ requires C, 68.9; H, 8.1%), ν_{\max} 3 590, 3 420br, 1 775, and 875 cm^{-1} , δ 1.09 (3 H, s), 1.19 (3 H, d, J 7 Hz), 1.32 (3 H, s), 2.31 (1 H, d, J 4 Hz), 3.68 (1 H, q, J 6 and 11 Hz), 4.84 (1 H, q, J 4 and 6 Hz), 6.28br (1 H, s), 7.25br (1 H, s), and 7.36br (1 H, s), m/e 348, 330, 246, 235, 208, 207, 181, 123, 109, 95, and 81.

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