Structures of Three New Diterpenoids from *Ballota* Species

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Ballonigrin and ballonigrinone, isolated from *B. rupestris*, have been shown to be 15,16-epoxy-7-oxolabda-8(9),13(16),14-trien-19,6β-olide (1) and the corresponding 3-ketone (2). 7α-Acetoxymarrubiin (5) has been isolated from *B. nigra*.

A NUMBER of furanoid diterpenoids of the labdane and clerodane series have been isolated from the *Labiatae*. In continuing our work on *Ballota* species,[1] we have examined *B. rupestris* and isolated two new related diterpenoids, ballonigrin, $C_{20}H_{24}O_4$, and ballonigrinone, $C_{20}H_{22}O_5$. Ballonigrin has also been isolated from *B. nigra*. The oxygen atoms of ballonigrin (1) were accounted for in a γ-lactone group ($\nu_{max.}$ 1 770 cm^{-1}), an αβ-unsaturated ketone system [$\nu_{max.}$ 1 680 cm^{-1}; $\lambda_{max.}$ 260 nm (ε 7 600)] and a furan ring ($\nu_{max.}$ 875 cm^{-1}). Ballonigrinone exhibited an additional carbonyl band at 1 700 cm^{-1}, ascribed to a cyclohexanone. The ^1H n.m.r. spectrum (see Table 1) of ballonigrin contained resonances assigned to two tertiary methyl groups, an olefinic methyl group, and three protons form a β-substituted furan ring. In addition there were a pair of AX doublets (J 6.0 Hz), one of which (δ 4.70) was assigned to the proton attached to the γ-lactone ring. Decoupling showed that this proton possessed an equatorial–axial relationship with a methine proton (δ 2.18) which itself was not further coupled. The noise-decoupled and single-frequency off-resonance decoupled ^{13}C n.m.r. spectra of ballonigrin (see Table 2) confirmed the presence of three methyl groups and showed that the remaining carbon atoms included five methylene, five methine, and seven quaternary carbon atoms. In the spectrum of ballonigrinone one of the methylene triplets was replaced by a carbonyl singlet. The fully substituted nature of the αβ-unsaturated ketone, implicit in the u.v. maximum, was confirmed by the presence of singlet resonances at δ 131.2 (α-C) and 166.7 (β-C) in the ^{13}C n.m.r. spectrum. The relationship of the lactone ring with the unsaturated ketone system was established by reduction of the carbonyl group with sodium borohydride to afford an oily

[1] G. Savona, F. Piozzi, J. R. Hanson, and M. Siverns, *J.C.S. Perkin I*, 1976, 1607.

alcohol (7). The ^{1}H n.m.r. spectrum of this compound contained a new doublet [δ 4.48 (*J* 9.0 Hz, C*H*·OH)], and the lactonic CH·O signal (δ 4.90) was now a quartet (*J* 5.6 and 9.0 Hz). Hence the carbonyl group was adjacent to the lactone ring. Since the methine proton signal (δ 2.18) was a simple doublet, the methine carbon atom must have two fully substituted carbon atoms attached to it. This led to assignment of the part structure (A), which may only be accommodated within a labdane as in structure (1). In confirmation of this structure, mild dehydration of 7-oxomarrubiin (4) [1] afforded ballonigrin (1). This raised the possibility that ballonigrin might be an artefact, but it may be detected by t.l.c. in the crude plant extract prior to purification.

TABLE 1

^{1}H N.m.r. signals (in $CDCl_3$; p.p.m. from Me_4Si)

Compound	20-H	18-H	17-H	5-H (*J*/Hz)	6-H (*J*/Hz)	7-H (*J*/Hz)	14-H	15-H	16-H
(1)	1.08	1.32	1.82	2.18 (6.0)	4.70 (6.0)		6.28	7.37	7.26
(7)	1.22	1.32	1.82	1.95 (5.5)	4.92 (5.6, 9.0)	4.48 (9.0)	6.29	7.37	7.26
(2)	1.04	1.54	1.87	2.18 (6.0)	4.64 (6.0)		6.22	7.37	7.24
(8)	1.30	1.51	1.78	1.95 (5.5)	4.96 (5.5, 9.0)	4.50 (9.0)	6.27	7.35	7.24 [a]
(5)	1.20	1.35	1.15 [b]	2.35 (4.2)	4.82 (4.2, 6.0)	5.50 (7.5, 6.0)	6.30	7.38	7.25 [c]
(6)	1.15	1.32	1.05 [b]	2.20 (4.5)	4.90 (m)	4.90 (m)	6.30	7.35	7.25 [d]

[a] 3-H, 3.63 (m). [b] Doublet, *J* 8 Hz. [c] Acetate, 2.18. [d] Acetate, 2.12.

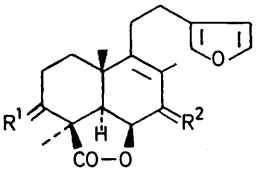
TABLE 2

^{13}C N.m.r. signals (in $CDCl_3$; p.p.m. from Me_4Si)

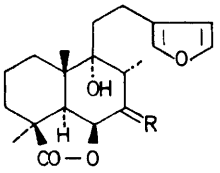
Carbon atom	Ballonigrin	Ballonigrinone	7α-Acetoxymarrubiin	7β-Acetoxymarrubiin
1	30.3	30.3	33.4	34.9
2	17.9	34.1	17.5	20.9
3	27.8	203.5	29.3 [a]	28.4 [a]
4	42.0	52.2	43.7	44.0
5	49.4	50.2	46.0	45.3
6	75.5	74.6	75.8	74.2
7	193.2	191.4	70.6	73.7
8	131.2	132.6	38.8	37.7
9	166.7	163.8	79.2	77.4
10	36.7	36.2	39.1	39.6
11	24.1 [a]	24.2 [a]	28.4 [a]	28.3 [a]
12	30.0 [a]	30.2 [a]	18.4	18.0
13	123.7	123.5	124.6	124.7
14	110.6	110.5	110.7	110.6
15	138.8 [b]	138.9 [b]	138.8 [b]	138.6 [b]
16	143.1 [b]	143.3 [b]	143.2 [b]	143.1 [b]
17	24.5	24.2	13.9	12.1
18	27.9	21.6	22.8 [c]	22.3 [c]
19	180.2	172.2	183.1	183.1
20	12.0	12.2	22.0 [c,d]	22.6 [c,d]

[a–c] These assignments may be interchanged. [d] Acetate, 21.0 and 171.0.

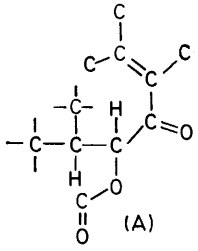
The ^{13}C n.m.r. resonances of ballonigrin were assigned by comparison with those of marrubiin (3).[2] In particular the spectrum lacked the doublet associated with C-8 in marrubiin, and the doublet associated with C-5 had moved downfield. The triplets associated with C-2 and C-3 remained relatively unaffected. In the spectrum of ballonigrinone a triplet resonance at δ 27.8 was replaced by a singlet at δ 203.5 (the carbonyl group), and another triplet at δ 17.9 was displaced downfield to 34.1. In addition the singlet at 42.0 had moved downfield to 52.5. The carbonyl group was therefore flanked by a methylene and a quarternary carbon atom. The resonances are those assigned to C-2, -3, and -4. Furthermore the chemical shift differences between ballonigrin and ballonigrinone



(1) $R^1 = H_2$, $R^2 = O$
(2) $R^1 = R^2 = O$
(7) $R^1 = H_2$, $R^2 = H, OH$
(8) $R^1 = R^2 = H, OH$



(3) R = H_2
(4) R = O
(5) R = H, OAc
(6) R = OAc, H



(A)

for the ring A carbon atoms closely parallel those between marrubiin and peregrinone [1,2] and between gibberellin A_9 and gibberellin A_4 3-ketone.[3] Thus the additional carbonyl group in ballonigrinone was located at C-3.

The mass spectra of ballonigrin and ballonigrinone had strong fragment ions at *m/e* 81 and 95, associated with alkylfurans. Another ion at *m/e* 109, also found in the spectra of marrubiin (3) and the kaurenolides,[4] is associated with a ring A fragment.

The third new diterpenoid, $C_{22}H_{30}O_6$, isolated from *B. nigra*, showed $\nu_{max.}$ 3 450 (OH), 1 780 (γ-lactone), 1 725 and 875 cm^{-1} (furan). The ^{1}H and ^{13}C n.m.r. spectra

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

[3] I. Yamaguchi, N. Takahashi, and K. Fujita, *J.C.S. Perkin I*, 1975, 992; R. Evans, J. R. Hanson, and M. Siverns, *J.C.S. Perkin I*, 1975, 1574.

[4] J. R. Hanson, G. Savona, and M. Siverns, *J.C.S. Perkin I*, 1974, 2001.

(see Tables) revealed the presence of an acetoxy-group and suggested that the diterpenoid was 7α-acetoxymarrubiin (5), and thus related to ballotinone (4). In particular the 6-H signal was now a quartet (J 4.2 and 6.0 Hz), and there was a new C*H*·OAc signal, also a quartet (J 6.0 and 7.5 Hz). The magnitudes of the 6-H,7-H and 7-H,8-H coupling constants indicated that the acetoxy-group had the α-configuration. Reduction of ballotinone with sodium borohydride and acetylation of the product gave the epimeric 7β-acetate. Reduction occurred from the less hindered α-face of the molecule.

EXPERIMENTAL

General details have been described previously.[1]

Extraction of Ballota rupestris.—*B. rupestris* Vis. was collected on Mount Pellegrino near Palermo in June 1974. The sample was identified in the Institute of Botany, University of Palermo. Air-dried leaves (0.5 kg) 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 crude residue was chromatographed on a dry silica gel column (Merck; deactivated with 15% water). Elution with 30% ethyl acetate–light petroleum gave a crude crystalline fraction (0.5 g) which was rechromatographed to afford *ballonigrin* (1) (100 mg), m.p. 212°, $[\alpha]_D^{20}$ +2° (*c* 0.25 in $CHCl_3$) (Found: C, 73.0; H, 7.35. $C_{20}H_{24}O_4$ requires C, 73.2; H, 7.3%), *m/e* 328, 313, 300, 285, 203, 109, 95, and 81 (100%). Further elution with 50% light petroleum–ethyl acetate gave a crude fraction (150 mg) which was further crystallized to afford *ballonigrinone* (2), m.p. 217°, $[\alpha]_D^{20}$ −11° (*c* 0.46 in $CHCl_3$) (Found: C, 69.8; H, 5.4. $C_{20}H_{22}O_5$ requires C, 70.2; H, 6.4%), *m/e* 342, 327, 314, 299, 283, 203, 95, and 81 (100%).

Reduction of Ballonigrin.—Ballonigrin (40 mg) was treated with an excess of sodium borohydride in methanol (5 ml) at room temperature for 1 h. The solution was acidified and concentrated, and the product recovered in ethyl acetate. Evaporation afforded an oil (homogeneous by t.l.c.) $\nu_{max.}$ 3 400, 1 760, and 875 cm^{-1}. Similar treatment of ballonigrinone also gave an oil, $\nu_{max.}$ 3 400, 1 760, and 875 cm^{-1} (for ^1H n.m.r. spectra see Table 1).

Dehydration of 7-Oxomarrubiin (4).—7-Oxomarrubiin (20 mg) in chloroform (3 ml) was warmed with alumina (300 mg) overnight. The solution was filtered and evaporated and the alumina was stirred with fresh chloroform, which was then evaporated. The combined residues were purified by preparative layer chromatography to afford ballonigrin (4 mg), by its i.r. spectrum.

7α-Acetoxymarrubiin.—This *diterpenoid*, isolated from *B. nigra*, had m.p. 170° (Found: C, 67.5; H, 7.7. $C_{22}H_{30}O_6$ requires C, 67.7; H, 7.7%), *m/e* 390, 330, 312, 249, 236, 207, 163, 123, 109 (100%), 95, and 81. *7β-Acetoxymarrubiin*, prepared by acetylation of 7-hydroxymarrubiin,[1] had m.p. 158° (Found: C, 67.4; H, 7.5%), $\nu_{max.}$ 3 500, 1 780, 1 725, and 875 cm^{-1}, *m/e* 390, 330, 312, 249, 218, 207, 181, 197, 163, 123, 109 (100%), 95, and 81.

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