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THE STRUCTURE AND CHEMISTRY OF PEBROLIDE, DESACETYLPEBROLIDE AND 1-DEOXYPEBROLIDE, SESQUITERPENE BENZOATES FROM *PENICILLIUM BREVICOMPACTUM*

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Abstract—The structure of three new drimane sesquiterpenes (**1**–**3**) has been established from chemical and spectroscopic evidence and by single crystal X-ray crystallographic analysis of **7**. Ring B in the crystal of **7** is in a chair conformation, slightly distorted because of *cis* fusion to the lactone ring and because three β axial substituents are present. NMR evidence suggests that the preferred conformation in solution is similar.

The fungus *Penicillium brevicompactum* is known to produce mycophenolic acid (**1**) and various metabolites related to it,[1] and evidence has accumulated that the side chain of these is derived from farnesyl pyrophosphate.[2] We now report details of the isolation from a strain of this fungus of three sesquiterpene benzoates, namely pebrolide (**2**), desacetylpebrolide (**3**) and 1-deoxypebrolide (**4**).[3]

These metabolites were obtained by chromatography of the neutral fraction of broth extracts of *P. brevicompactum* grown in surface culture. Pebrolide (**2**) formed needles, $C_{24}H_{30}O_7$, m.p. 167–170°, $[\alpha]_D$ −41° ($CHCl_3$), and showed spectral features consistent with presence of two secondary OH functions (one free and one benzoylated), two primary OH functions (one acetylated and the other as part of a γ-lactone system) and two tertiary Me groups. Acetylation and oxidation gave respectively the diacetate **5** and the 6-membered ring ketone **6**. The complete structure of **2** was established by X-ray crystallographic analysis of the bromoacetate **7** as described later.

The second metabolite, desacetylpebrolide (**3**), showed spectral features similar to those of **2** but lacked the characteristics of an acetate group. Acetylation afforded the diacetate **5** previously obtained from **2** and final structural proof was provided by preparation of **3** in good yield by selective hydrolysis of the acetate group in **2** using dilute acid. Hydrolysis of the acetate group in **2** using base was accompanied by isomerization of the axial 8β-lactonic substituent to equatorial 8α-, forming the less crowded *trans* fused lactone **8** and this was also obtained from **3** under the same conditions (in less than a min). Analogous epimerizations have been reported for the *cis*-lactones dihydroiresin (**9**)[4] and dihydroconfertifolin (**10**).[5] These epimerizations are accompanied by characteristic changes in the NMR in the region 4–5 δ as follows.

The signals for H-11α and H-11β in the spectrum of **3** (and in the spectrum of **2**) appear as a double doublet at 4.26 ppm and a doublet at 4.98 ppm respectively. The former signal collapses to doublets J = 9.5 Hz and J = 5.5 Hz upon irradiation at *ca* 2.52 ppm (corresponding to H-9) and *ca* 4.98 ppm respectively, confirming that coupling between H-9 and H-11β is negligible. In a model of **2** in which ring B has a slightly distorted chair conformation, dihedral angles between H-9 and H-11α and H-11β are *ca* 30° and 90° giving calculated[6] coupling constants close to those observed. Although the corresponding proton signals in the spectrum of **10**[5] appear as a multiplet in $CDCl_3$, this is spread out in benzene to show the same splitting pattern and coupling constants as **2** and **3** (*cf* Table 1). By contrast, in the spectra of the *trans*-lactones **8** and **11** (*trans*-dihydroconfertifolin), the signals for the protons at C-11 and C-9 form an unexceptional ABX system with values of J_{AX} and J_{BX} (*cf* Table 1) in agreement with values calculated from dihedral angles of *ca* 35° and 157°. The chemical shift values in these isomers are discussed later.

The third metabolite, 1-deoxypebrolide (**4**), m.p. 171–173°, was obtained in much smaller amounts than **2** or **3** and differed from **2** only in the absence of the secondary OH group as indicated by the appropriate

Table 1. δ_H in $CDCl_3$ (J)

Compound	Nucleus				
	H-6	H-11α	H-11β	H-13	H-14
2	5.72	4.26 (5.5, 9.5)	4.98 (9.5)	1.44	0.96
3	5.75	4.26 (5, 9.4)	4.98 (9.4)	1.47	0.88
	6.03*	4.28*	5.30*	1.80*	1.00*
4	5.70	4.18 (5.5, 9.5)	4.38 (9.5)	1.40	0.96
5	5.72	4.22 (5.5, 9.5)	4.64 (9.5)	1.56	1.00
6	5.75	4.23 (5.4, 9.8)	4.45 (9.8)	1.57	1.10
7	5.70	4.28 (5.5, 10)	4.75 (10)	1.59	1.01
8	5.82	4.55 (7, 8)	4.20 (8, 12)	1.45	0.94
	6.15*	4.71*	4.34*	1.62*	1.02*
10		4.12 (5.5, 10)	4.22 (10)	0.85	0.79
		3.45†	3.72†	0.75†	0.64†
11		4.21 (7.5, 9)	3.95 (9, 12)	0.97	0.86
12	4.44	4.11 (7, 8)	4.09 (8, 12)	1.25	1.13
13	5.35	4.24 (5.3, 10)	4.94 (10)	1.33	1.01
14	5.50	4.27 (5.3, 10)	4.95 (10)	1.35	0.90

* In C_5D_5N † In C_6D_6

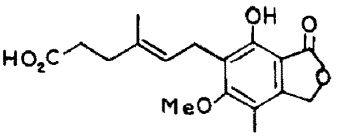
spectral differences and similarities. Basic hydrolysis was again accompanied by isomerization at C-8, giving the dihydroxy *trans*-lactone **12**.

In keeping with the equatorial configuration of the OH group at C-1 in **2**, catalytic reduction of **6** using Pt in HOAc afforded the cyclohexanecarboxylate derivative **13**, which was also obtained from **2**. Under the same conditions a similar dervative **14** was obtained from **3**.

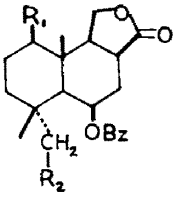
Although it might seem from models that the steric congestion of the three axial substituents in **2** and **3** (which explains the facile epimerization observed) would be relieved if ring B adopted a twist boat conformation, this would not give appropriate dihedral angles for the protons at C-9 and C-11. Hence, for **2** in solution, ring B is a chair as in the crystal of its bromoacetate **7**. Shielding effects depending on proximity to the oxygen functions at C-1 (on H-11β), C-6 (on H-13) and C-15 (on H-6) can be interpreted on the basis of this conformation for ring B. Comparison of the spectrum of **2** (or **3**) with those of **4** and **10** shows a downfield shift for H-11α of 0.08 and 0.14 ppm and for H-11β of 0.60 and 0.76 ppm. In **8** the shift difference for these protons relative to **11** are 0.34 and 0.25 ppm respectively. This reflects the fact that in the *cis* compounds H-11β is much closer than H-11α to the O atom at C-1 whereas in the *trans* compounds the distances are more nearly equal. The shielding effect is slightly reduced upon esterification of the OH group (see **5** and **7** in Table 1), but markedly accentuated by complexing with pyridine[8] (see values for **3** and **8** in pyridine).

Similar considerations apply to the Me signals at 1.44 and 0.96 ppm in the spectrum of **2** which can be assigned to H-13 and H-14 respectively on the basis of esterification shifts or via correlation with the ^{13}C NMR signals (11.7 and 19.6 ppm respectively) using SFORD residual couplings. The axial OH group at C-6 in **12** is roughly equidistant from C-13 and C-14 and comparison of the ^1H NMR spectra of **11** and **12** shows downfield shifts of *ca* 0.3 ppm in both signals. However comparison of the spectra of the various 6-acyloxy compounds e.g. the *cis* compounds **3** and **4** with that of **10** or the *trans* compound **8** with that of **11** indicates that only the C-13 proton signals are shifted significantly downfield (0.43 0.50 ppm) by the net effect of the acyloxy grouping. Because of the axial substituents at C-4 and C-8, the 6-acyloxy substituent would be expected to exist largely as a rotamer similar to that found in the crystal of **7** in which the CO group, O-6 and C-13 are aligned with the carbonyl O atom remote from C-13. Thus the C-13 and C-14 protons could lie in the deshielding and shielding zones of the CO group respectively. If, in addition, the C-13 and C-14 protons are both deshielded by the lone pair on the O atom at C-6 (both signals in **3** and **8** show $CDCl_3$-pyridine downfield shifts), the net effect of the 6-acyloxy group will be as observed.

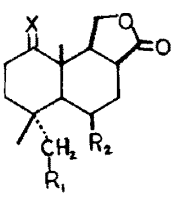
It is also evident from Table 1 that the oxygen substituent at C-15 in **2** and its derivatives deshields H-6. The absence of any significant $CDCl_3$-pyridine shift of the Me group at C-4 in some diterpenoids having a 4α-CH_2OH group has been used as evidence that the latter has a preferred conformation in which the OH



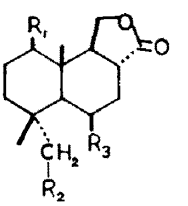
1.



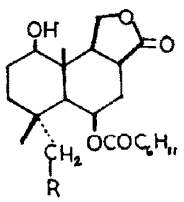
2. R_1 = OH, R_2 = OAc
3. R_1 = R_2 = OH
4. R_1 = H, R_2 = OAc
5. R_1 = R_2 = OAc
7. R_1 = $OCOCH_2Br$, R_2 = OAc



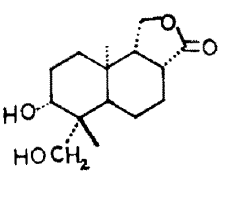
6. X = O, R_1 = OAc, R_2 = OBz
10. X = H_2, R_1 = R_2 = H



8. R_1 = R_2 = OH, R_3 = OBz
11. R_1 = R_2 = R_3 = H
12. R_1 = H, R_2 = R_3 = OH



13. R = OAc
14. R = OH



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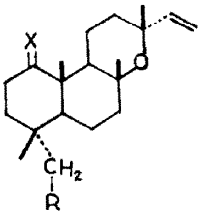

15. X = O, R = H
16. X = H_2, R = H
17. X = β-OH, αH; R = OAc

group is antiperiplanar to the axial C-4 Me group.[9] This would also seem to apply to C-14 in 3 which shows a $CDCl_3$-pyridine shift of only 0.1 ppm. In the crystal of 7, torsion angles associated with the acetoxymethyl group are 72° for C(3)-C(4)-C(15) O(15) and −3° for C(15) O(15)-C(1″)-O(1″) so that O(15) is indeed antiperiplanar to C(13). If this were the preferred orientation in solution, the CO group would be unlikely to have a significant shielding effect on H-6.

The absolute configuration of these metabolites as determined for 7 by crystallography[10] could also be deduced from ORD data. The ORD curves of **2** and **4** were very similar. A difference curve between **4** and **6** shows a small positive Cotton effect as would be predicted on the basis of the Octant rule[11] or by analogy with 1-ketomanoyl oxide **15**.[12]

EXPERIMENTAL

Isolation of the metabolites **2**–**4**. The filtrates from 28-day old surface cultures of a strain of *Penicillium brevicompactum* grown on Czapek-Dox 1% corn steep liquor were stirred with charcoal (10 g/l.) for 1–2 hr and the crude mixture of metabolites recovered from this by Soxhlet extraction with acetone. Chromatography of the neutral fraction of this mixture on silica afforded deoxypebrolide (**4**, eluted with 10% $CHCl_3$ in benzene; *ca* 4 mg/l.), pebrolide (**2**, eluted with $CHCl_3$, *ca* 30 mg/l.) and desacetylpebrolide (**3**, eluted with 5% MeOH in $CHCl_3$; *ca* 45 mg/l.) together with metabolites related to mycophenolic acid.

Pebrolide (**2**). This crystallised from pet. ether-$CHCl_3$, m.p. 167–170°, R_f 0.65 on tlc with silica gel and MeOH $CHCl_3$ (1:9), $[\alpha]_D$ = −41° ($CHCl_3$); ORD $[\Phi]_{244}$ −1340, $[\Phi]_{258}$ (trough) −3950, $[\Phi]_{284}$ −1220, $[\Phi]_{333}$ −465, $[\Phi]_{400}$ −60; UV λ_{max} 230 nm (ε 9,700); IR (KBr) 3500, 1764, 1710, 1597, 1580, 1243, 711 cm^{-1}; IR ($CHCl_3$) 3605, 1780 (lactone, ε 1110), 1740 (acetate, ε 620), 1715 cm^{-1} (benzoate, ε 910); ^1H NMR ($CDCl_3$) 2.04 (3 H, s, OAc), 3.30 (1 H, m, H-1), 3.79 and 3.97 (ea. 1 H, ABq, J = 12 Hz, CH_2OAc), 7.50 and 8.04 (3 H and 2 H, m, benzoate); ^{13}C NMR ($CDCl_3$) C-1 to C-15 at 82.4, 27.4, 35.1, 36.8, 46.4, 67.6, 28.0, 35.9, 48.1, 39.3, 70.1, 178.4, 11.7, 19.6, 72.0 ppm respectively, also acetate at 27.3, 170.8 ppm, benzoate at 128.5 (2C), 129.7 (2C), 130.3 (C attached to CO_2R) and 133.0 ppm;† MS *m/e* 430 (0.1% M^+).

†^{13}C NMR assignments for **2** were made using the SFORD spectrum and by agreement with values estimated by applying appropriate substituent increments[13] to values for **10** (see below). The increments due to the oxygen substituents at C-1 and C-15 were taken as the difference in values for manoyl oxide (**16**) and jhanidiol-18-monoacetate (**17**),[14] namely, for C-1 to C-10: 40.0, 10.3, −8.1, 2.9, −7.3, 0.3, −0.6, −0.3, 0.3, 5.8 and for C-13 to C-15: −3.7, −4.5 and 39.1 ppm respectively. ^{13}C NMR of **10** ($CDCl_3$): C-1 to C-15 at 40.4, 18.1, 42.0, 32.9, 51.4, 18.4, 22.4, 37.4, 49.9, 35.4, 67.6, 179.1, 14.5, 22.0 and 33.5 ppm respectively. (Assignments again using the SFORD spectrum and by agreement with values estimated by applying appropriate substituent increments[13] to values for the corresponding trimethyl *trans*-decalin[15]).

357 (5), 325 (10, M-105), 308 (30, M-122), 266 (40, M-122-42), 265 (70, M-105 − 60), 248 (20, M-122-60), 247 (18), 235 (40), 230 (30), 217 (50), 105 (100) with m* corresponding to 325 → 265, 308 → 290, 266 → 248, 265 → 247, 248 → 230 and 235 → 217. (Found: C, 66.6; H, 7.0. $C_{24}H_{30}O_7$ requires: C, 67.0; H, 7.0%).

Desacetylpebrolide (**3**). Isolated as above, this crystallised from $CHCl_3$-pet. ether, m.p. 252–255°, R_f 0.28 using the same tlc system as for **2**, $[\alpha]_D = -25$ ($CHCl_3$), ORD $[\Phi]_{248} - 455$, $[\Phi]_{259}$ (trough) -3020, $[\Phi]_{285} - 650$, $[\Phi]_{333} - 420$, $[\Phi]_{400} - 260$: UV λ_{max} 230 nm (ε 11,900); IR (KBr) 3420, 1756, 1713, 1602, 1582, 711 cm^{-1}; IR ($CHCl_3$) 1771 (γ-lactone, ε 540), 1711 cm^{-1} (benzoate, ε 470); ^{1}H NMR (C_5D_5N), 3.54 (1 H, m, H-1), 3.26 and 3.84 (ea. 1 H, ABq, J = 11 Hz, CH_2OH); MS m/e 388 (0.1%, M$^+$), 358 (1), 357 (1, M-31), 283 (25, M-105), 266 (15, M-122), 248 (3), 236 (20), 218 (12), 192 (10), 105 (100) with m* corresponding to 388 → 299, 388 → 266, 358 → 304, 266 → 235 and 236 → 218. (Found: C, 68.4; H, 7.1 $C_{22}H_{28}O_6$ requires: C, 68.0; H, 7.3%).

1-Deoxypebrolide (**4**). Isolated as above, this crystallized from ether, m.p. 171–173°, R_f 0.87 using same tlc system as for **2**; ORD $[\Phi]_{244} - 220$, $[\Phi]_{260}$ (trough) -460, $[\Phi]_{284} - 1410$, $[\Phi]_{400} - 110$; IR (KBr) 1775, 1738, 1710, 1605, 1588, 1249, 711 cm^{-1}; IR ($CHCl_3$) 1783 (γ-lactone, ε 780), 1735 (acetate, ε 600) 1717 cm^{-1} (benzoate, ε 730); ^{1}H NMR ($CDCl_3$), 2.04 (3 H, s, OAc), 3.77 and 3.97 (ea. 1 H, ABq, J = 12 Hz, CH_2OAc), 7.50 and 8.00 (3 H and 2 H, m, benzoate); MS m/e 414 (0.1%, M$^+$), 292 (5, M-122), 258 (30), 249 (30, M-105-60), 232 (10, M-122-60), 219 (10, M-122-73), 167 (100), 105 (50) with m* corresponding to 292 → 219. (Found: C, 69.6; H, 7.3. $C_{24}H_{30}O_6$ requires: C, 69.6; H, 7.2%).

O-Acetylpebrolide (**5**). Acetylation of either **2** or **3** using Ac_2O-pyridine under standard conditions gave **5**, m.p. 178–180° from ether: IR (KBr) 1781, 1739, 1709, 1599, 1580, 1247, 711 cm^{-1}; ^{1}H NMR ($CDCl_3$) 2.08 and 2.11 (ea. 3 H, s, OAc), 3.81 and 4.01 (ea. 1 H, ABq, J = 12 Hz, CH_2OAc), 4.64 (1 H, m, H-1), 7.56 and 8.04 (3 H and 2 H, m, benzoate); MS m/e 472 (1%, M$^+$), 399 (8), 367 (7, M-105), 350 (30, M-122), 308 (50, M-122-42), 307 (48, M-105-60), 290 (45, M-122-60), 265 (12, M-105-60-42), 247 (25, M-105-60-60), 235 (25), 230 (70, M-122-60-60), 217 (90), 105 (100) with m* corresponding to 367 → 307, 307 → 247, 307 → 265, 290 → 230, 265 → 217, and 235 → 217. (Found: C, 65.8; H, 6.9. $C_{26}H_{32}O_8$ requires: C, 66.1; H, 6.8%).

The ketone **6**. Pebrolide **2** (106 mg) in acetone was oxidised with a slight excess of CrO_3 in H_2SO_4 for 1 min. After pouring into ice water, extraction with $CHCl_3$ gave **6** (94 mg, 89%), m.p. 187–190°, ORD $[\Phi]_{255}$ (trough) -404, $[\Phi]_{296}$ 0, $[\Phi]_{315}$ (peak) $+430$, $[\Phi]_{321}$ 0, $[\Phi]_{400} - 215$;† IR (KBr) 1787, 1732, 1711, 1598, 1582, 711 cm^{-1}; IR ($CHCl_3$) 1778 cm^{-1} (γ-lactone, ε 680), 1736 (acetate, ε 520), 1710 (ketone, benzoate, ε 850); ^{1}H NMR ($CDCl_3$), 2.03 (3 H, s, OAc), 3.84 and 4.02 (ea. 1 H, ABq, J = 11 Hz, CH_2OAc), 7.35 and 7.85 (3 H and 2 H, m, benzoate); MS m/e 428 (0.1%, M$^+$), 323 (10, M-105), 306 (20, M-122), 263 (30, M-105-60), 246 (10, M-122-60), 233 (8), 223 (9), 197 (15), 105 (100) with m* corresponding to 428 → 323 and 323 → 263 (Found: C, 67.0; H, 6.6. $C_{24}H_{28}O_7$ requires: C, 67.3; H, 6.5%).

O-Bromoacetylpebrolide (**7**). Pebrolide **2** (24 mg) in benzene (10 ml) containing pyridine (5 drops) was allowed to stand with $BrCH_2COBr$ (1 ml) for 1 hr. After filtration, evaporation gave an oil which was taken up in $CHCl_3$, washed with aq. $NaHCO_3$ and water. Evaporation gave the *bromoacetate* **7** (10 mg, 30%), m.p. 150–151°, as prisms from ether; IR (KBr) 1780, 1730, 1607, 1590, 1250, 720 cm^{-1}, ^{1}H NMR ($CDCl_3$) 2.08 (3 H, s, OAc), 3.86 (2 H, s, $BrCH_2CO_2$–), 3.83 and 4.00 (ea. 1 H, ABq, J = 11 Hz, CH_2OAc), 5.00 (1 H, m, H-1), 7.46 and 8.05 (3 H and 2 H, m, benzoate); MS m/e 552 (550) [1%, M$^+$], 447 (445) [1, M-105], 430 (428) [5, M-122], 387 (385) [25, M-105-60], 307 (15, M-105-140 (138)], 290 [12, M-122-140 (138)], 230 [40, M-122-140 (138)-60], 217 [50, M-122-140 (138)-73], 105 (100).

Hydrolysis of **2**. Pebrolide **2** (18 mg) in acetone was treated with 6N H_2SO_4 (2 ml). After 24 hr at room temp, extraction with $CHCl_3$ afforded **3** (12 mg, 74%), m.p. 252–255° from $CHCl_3$-pet. ether, identical with the isolated material (mixed m.p., IR and NMR spectra).

Isodesacetylpebrolide (**8**). Desacetylpebrolide **3** (19 mg) in an excess of methanolic KOH was allowed to stand for 3 hr and then neutralised with 5N HCl. Extraction with $CHCl_3$ gave **8** (17 mg, 89%), m.p. 192–193° from ether; IR (KBr) 3460, 1760, 1718, 1607, 1590, 717 cm^{-1}; IR ($CHCl_3$) 1778 cm^{-1} (γ-lactone, ε 710), 1719 (benzoate, ε 610); ^{1}H NMR ($CDCl_3$) 3.42 (1 H, m, H-1), 3.63 and 3.17 (ea. 1 H, ABq, J = 12 Hz, CH_2OH), 7.57 and 8.10 (3 H and 2 H, m, benzoate); MS m/e 388 (0.1%, M$^+$), 358 (1), 357 (1, M-31), 283 (25, M-105), 266 (15, M-122), 248 (3, M-122-18), 236 (20, M-122-30), 235 (13, M-122-31), 218 (12), 192 (10), 105 (100) with m* corresponding to 388 → 266, 266 → 248, 266 → 235, and 236 → 218. (Found: C, 68.3; H, 7.4. $C_{22}H_{28}O_6$ requires: C, 68.0; H, 7.3%).

This product was also obtained by similar treatment of **2**.

The diol **11**. 1-Deoxypebrolide **4** (34 mg) in MeOH was refluxed with 5N NaOH (5 ml) for 3 hr. After neutralization with dil. HCl, extraction with $CHCl_3$ gave benzoic acid (11 mg) together with the *diol* **11** (16 mg, 73%), m.p. 149–154° from $CHCl_3$-pet. ether: IR (KBr) 3500, 1745 cm^{-1}; IR ($CHCl_3$) 3610 cm^{-1} (OH, ε 200), 1770 (γ-lactone, ε 490); ^{1}H NMR ($CDCl_3$), 3.25 and 3.47 (ea. 1 H, ABq, J = 11 Hz, CH_2OH); MS m/e 268 (1%, M$^+$), 250 (2, M-18), 237 (30, M-31), 232 (10, M-18-18), 221 (5), 220 (17), 219 (100, M-31-18), 191 (15), 173 (20) with m* corresponding to 237 → 219, 219 → 201. (Found: C, 66.9; H, 8.8 $C_{15}H_{24}O_4$ requires: C, 67.1, H, 9.0%).

Hexahydropebrolide (**13**). (i) The ketone **6** (30 mg) in HOAc (10 ml) with PtO_2 (20 mg) was hydrogenated for 3 hr. After filtration through glass paper and evaporation of the solution, crystallization from ether gave the *ester* **13** as plates (10 mg), m.p. 165–167°; IR (KBr) 3500, 1760, 1730, 1260 cm^{-1}; IR ($CHCl_3$) 1780 cm^{-1} (γ-lactone, ε 667), 1732 (acetate, cyclohexane carboxylate, ε 764); ^{1}H NMR ($CDCl_3$) 3.3 (1 H, m, H-1), 3.9 and 3.78 (ea. 1 H, ABq, J = 12 Hz, CH_2OAc); MS m/e 436 (1%, M$^+$), 325 (20, M-111), 309 (40, M-127), 308 (45, M-128), 265 (100, M-111-60), 249 (45, M-127-60), 235 (30, M-128-73) with m* corresponding to 309 → 249 and 308 → 235. (Found: C, 65.9; H, 8.4. $C_{24}H_{36}O_7$ requires: C, 66.0; H, 8.3%) By contrast, the ketone **6** was recovered unchanged after hydrogenation in EtOH with 5% Pd-C.

(ii) Reduction of pebrolide **2** (80 mg) under similar conditions with PtO_2 (50 mg) gave **13** (72 mg, 89%), m.p. and m.m.p. 165–167°; R_f and IR spectrum as for previous sample.

Hexahydrodesacetylpebrolide (**14**). Desacetylpebrolide (**3**, 160 mg) in HOAc (14 ml) with PtO_2 (144 mg) was hydrogenated for 20 hr. After the usual work up the *ester* **14** was obtained as plates (152 mg, 92%), m.p. 210–215° from ether; IR (KBr) 1740, 1715 cm^{-1}; IR ($CHCl_3$) 1772 cm^{-1} (γ-lactone, ε 1000), 1717 (cyclohexanecarboxylate, ε 621); ^{1}H NMR ($CDCl_3$) 3.14 and 3.58 (ea. 1 H, ABq, J = 11 Hz, CH_2OH); MS m/e 394 (1%, M$^+$), 363 (3, M-31), 283 (100, M-111), 267 (80, M-127), 266 (85, M-128), 249 (30, M-127-18), 248 (30, M-128-18), 237 (50), 236 (75), 235 (70, M-128-31), 218 (50), 217 (75) with m* corresponding to 267 → 249, 266 → 248 and 266 → 235. (Found: C, 67.2, H, 8.8. $C_{22}H_{34}O_6$ requires: C, 67.0; H, 8.7%).

Crystallographic analysis of pebrolide bromoacetate (**7**).‡ A single crystal of **7**, grown from an ethereal soln, was mounted so as to rotate about the *a* axis. Oscillation, rotation and Weissenberg photographs were recorded using Cu-K_α radiation (λ = 1.5418 Å). Precession photographs were recorded using Mo-K_α radiation (λ = 0.7107 Å). A small crystal bathed in a uniform X-ray beam was used for intensity

†Subtraction of the curve for **4** gives a difference curve. $[\Phi]_{280} = -450$, $[\Phi]_{288} = 0$, $[\Phi]_{307}$ (peak) 1200, $[\Phi]_{334} = 0$, $[\Phi]_{340} = [\Phi]_{360} = -100$.

‡Crystallographic data and details of molecular geometry have been deposited with the Cambridge Data Centre

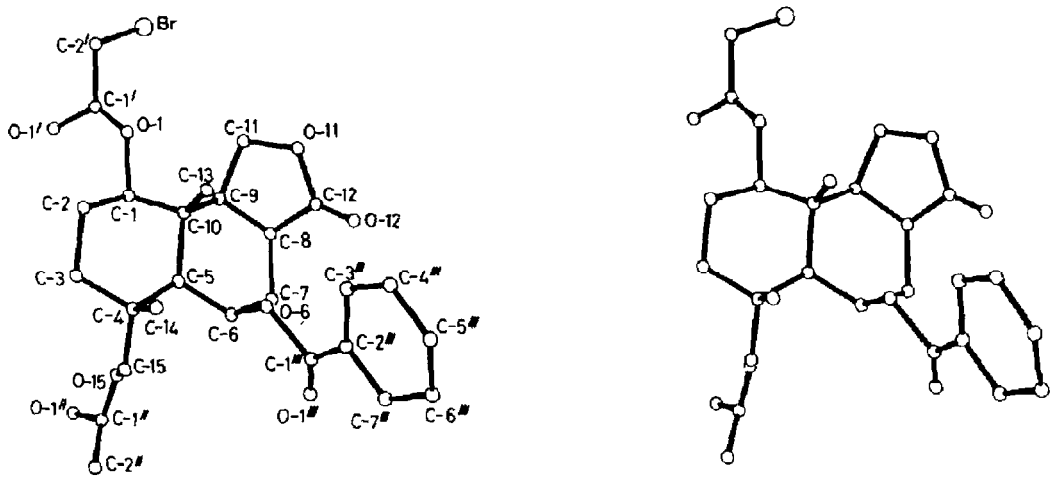

Fig. 1. ORTEP drawing giving stereoscopic view of pebrolide bromoacetate (7).

measurements. The data were collected on a Nonius camera using Robertson's multiple-film technique, reciprocal lattice nets 0 kl- 7 kl being recorded. Intensities were estimated using a Joyce-Lobel flying spot integrating microdensitometer, intensity values being corrected for appropriate Lorenz polarization and rotation factors. The various nets of F_o's were placed on an approximately absolute scale at a later stage of the refinement: 879 independent reflections were measured and used in the structure soln and refinement.

Crystal data. $C_{26}H_{31}BrO_8$, M = 552. Monoclinic, a = 9.08, b = 9.41, c = 15.16 Å, β = 98.2°, V = 1282 Å, z = 2, D_c = 1.43 g cm^{-3}. Space group $P2_1$ from systematic absences, OkO when k = 2n + 1.

Structure solution and refinement. The coordinates of the Br atom were found from 3-dimensional Patterson synthesis. In the first electron density distribution calculated with the observed structure amplitudes and the bromine phase angle, there was as expected, a false mirror plane. However it proved possible to select a few peaks as genuine atoms. Successive cycles of structure factor and electron distribution calculations allowed more and more atoms to be distinguished until after 7 cycles, the complete structure was revealed as 7. The R factor was 0.17. Structure factor least squares methods using programmes devised and written by J. G. Sime, D. W. Cruickshanks and J. G. F. Smith were used for the refinement process taking isotropic temperature factors U_{iso} of 0.05 for Br and C or O atoms respectively in the initial stages of the refinement.

Although no great accuracy can be claimed in this analysis, the final coordinates are sufficient to unambiguously establish the structure as 7. Bond lengths and atom densities suggest some disorder of the acetate group of the type $-O-C(=O)-Me \cdots O-C(-Me)=O$ but this in no way affects the validity of the structure. Rings A and B are both chairs but with a certain amount of distortion particularly in ring B which has the fused *cis*-lactone ring and three β axial substituents. A stereoscopic view of the molecule is shown in Fig. 1. The absolute configuration of 7 was determined by visual measurement of 6 Bijvoet pairs, and calculation of structure factors taking into account the anomalous dispersion corrections for Br in the International Tables.[10]

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