Tetranortriterpenoids and Related Substances. Part XVII.[1] A New Skeletal Class of Triterpenoids from *Guarea glabra* (Meliaceae)[2]

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Seven new triterpenoids from the heartwood of *Guarea glabra* are formulated as (1)—(5), (14), and (26) on the basis of chemical, spectroscopic, and *X*-ray evidence. All are based on the novel pentacyclic triterpane skeleton, (20*S*)-4,4,8β-trimethyl-14,18-cyclo-5α,13α,14α,17α-cholestane (16).

As part of our investigations [1] of the chemistry of the Meliaceae we examined an extract of the heartwood of *Guarea glabra* and isolated seven new triterpenoids (1)—(5), (14), and (26) which are derivatives of the novel pentacyclic triterpane (16). The nature of the carbon skeleton was established by *X*-ray analysis of the chloroacetate (18) and the iodoacetate (19) (see later).

The first compound, glabretal (1), $C_{32}H_{50}O_6$, was not obtained crystalline. Its n.m.r. spectrum readily reveals the presence of six tertiary methyl groups, an axial secondary acetate [δ 5·04 ($W_{\frac{1}{2}}$ 7 Hz, H-7)], an axial secondary alcohol [δ 3·46 ($W_{\frac{1}{2}}$ 8 Hz, H-3)], a trisubstituted epoxide [δ 2·88 (d, *J* 7 Hz, H-24)], a hemiacetal ring [δ 3·82 (m, H-23) and 5·45 (m, H-21 †)], and a cyclopropyl methylene group [δ 0·37 and 0·66 (ABq, *J* 5·5 Hz)]. Irradiation at δ 3·82 (H-23) caused the doublet at δ 2·88 (H-24) to collapse to a sharp singlet, thus demonstrating the vicinal nature of the protons involved. Oxidation of glabretal (1) afforded the keto-γ-lactone (15), m.p. 215—218° [$\nu_{max.}$ (CCl_4) 1782, 1736, and 1708 cm^{-1}], which, on reaction with sodium borohydride, yielded the oily triol (17) [$\nu_{max.}$ (CCl_4) 3620, 3520, and 1735 cm^{-1}] by reduction of both the cyclohexanone and γ-lactone functions. Acetylation of glabretal (1) followed by selective hydrolysis of the hemiacetal acetate on basic alumina and oxidation gave the acetoxy-γ-lactone (6), m.p. 220—222°.

These results indicate that glabretal is a pentacarbocyclic triterpenoid with a side chain similar to that found in turreanthin [3] and related compounds.[4] Initially, we considered the possibility that the *Guarea* compounds were cycloartane derivatives since cycloeucalenol and 24-methylenecycloartenol have been reported from the heartwood of *Swietenia* [5,6] and *Lovoa* [6] species, which are also members of the Meliaceae. It was soon apparent, however, that this was not the case since the o.r.d. of the

† Compounds (1)—(5) and (14) are mixtures of C-21 epimers.

[1] Part XVI, J. D. Connolly, I. M. S. Thornton, and D. A. H. Taylor, *J.C.S. Perkin I*, 1973, 2407.

[2] Preliminary communication, G. Ferguson, P. A. Gunn, W. C. Marsh, R. McCrindle, R. Restivo, J. D. Connolly, J. W. B. Fulke, and M. S. Henderson, *J.C.S. Chem. Comm.*, 1973, 159.

[3] C. W. L. Bevan, D. E. U. Ekong, T. G. Halsall, and P. Toft, *J. Chem. Soc.* (*C*), 1967, 820.

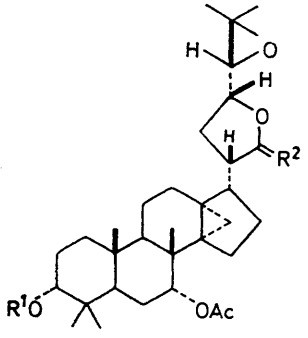
[4] J. D. Connolly, K. H. Overton, and J. Polonsky, *Progr. Phytochemistry*, 1970, **2**, 390.

[5] L. Amoros-Marin, W. I. Torres, and C. F. Asenjo, *J. Org. Chem.*, 1959, **24**, 411.

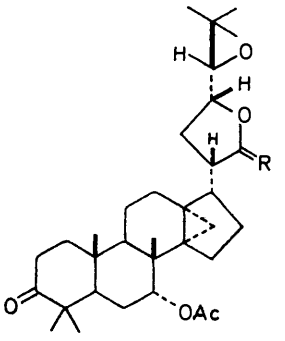
[6] K. L. Handa, Ph.D. Thesis, University of Glasgow, 1968.

second *Guarea* compound, the ketone (14) ([α] +25°), is markedly different from that of a 3-oxocycloartane ([α] −75°).[7] We concluded, therefore, that we were dealing with a new carbon framework and decided to settle the problem by *X*-ray analysis (see later).

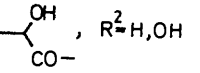
From its spectroscopic properties the second compound (14), $C_{32}H_{48}O_6$, m.p. 165—167°, is obviously closely related to (1). The presence of a band at 1709 cm^{-1} in the i.r. and the absence of the secondary hydroxy-proton in the n.m.r. suggest it is the corresponding ketone. This was readily established by oxidation of (14) to the keto-γ-lactone (15).

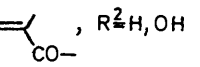


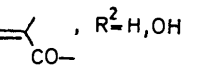
(1) R^1=H, R^2=H,OH

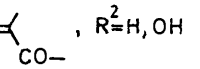


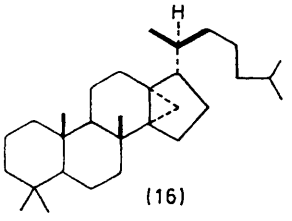
(14) R=H,OH
(15) R=O

(2) R^1=  , R^2=H,OH

(3) R^1=  , R^2=H,OH

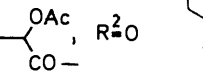
(4) R^1=  , R^2=H,OH

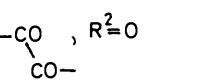
(5) R^1=  , R^2=H,OH



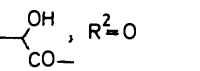
(16)

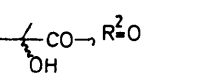
(6) R^1=Ac, R^2=O

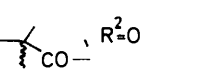
(7) R^1=  , R^2=O

(8) R^1=  , R^2=O

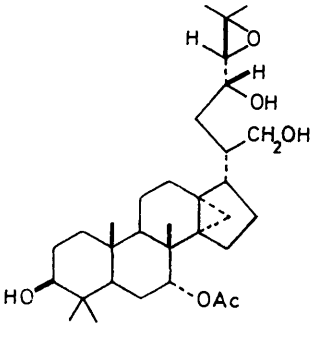
(9) R^1=H, R^2=O

(10) R^1=  , R^2=O

(11) R^1=  , R^2=O

(12) R^1=  , R^2=O

(13) R^1= $CH_3CO\cdot CO-$, R^2=O



(17)

* This was erroneously described as the acrylate in our preliminary communication.[2]

The third compound (2), $C_{37}H_{58}O_8$, m.p. 198—200°, is the 2-hydroxy-3-methylbutyrate of (1). It has signals in the n.m.r. at δ 0·90 (6H, d, *J* 7 Hz, Me_2CH), 3·95 (d, *J* 4 Hz, >C*H*OH), and 4·78br (s, H-3). On subjection to the acetylation, selective hydrolysis, and oxidation procedure described above, (2) was converted into the acetoxy-γ-lactone (7), m.p. 204—205° [δ 4·9 (d, *J* 4 Hz, >C*H*OAc)]. Oxidation of (2) itself afforded a mixture of two γ-lactones. The less polar is the α-keto-ester (8), m.p. 209—210° [$\nu_{max.}$ ($CHCl_3$) 1768 and 1720 cm^{-1}], whose n.m.r. spectrum readily confirms the presence of a 3-methyl-2-oxobutyrate grouping. The proton adjacent to the α-dicarbonyl system appears as a septet [δ 3·18 (*J* 7 Hz)] which is coupled to two secondary methyl groups [δ 1·16 (6H, d, *J* 7 Hz)]. The α-keto-ester function of (8) underwent ready hydrolysis in aqueous sodium hydrogen carbonate solution to give the hydroxy-γ-lactone (9), m.p. 202—204°, whose structure was confirmed by oxidation to the known keto-γ-lactone (15). The more polar oxidation product of (2) is the α-hydroxy-ester (10), m.p. 225—226°, which still retains the 2-hydroxy-3-methylbutyrate group [δ 4·06 (d, *J* 4 Hz, >C*H*OH)].

The fourth, fifth, and sixth compounds are the angelate (3), the tiglate (4), and the methacrylate (5).* These were isolated as a crystalline mixture, m.p. 137—139°, which could not be separated by t.l.c. on silver nitrate-impregnated plates. The mass spectrum indicates the presence of a mixture of homologues with peaks at *m/e* 612 ($C_{37}H_{56}O_7$) and 598 ($C_{36}H_{54}O_7$). From their other spectroscopic properties it is apparent that the three esters (3)—(5) have the side chain, the secondary acetate, and the cyclopropane ring in common with the first three compounds in this series and are C-3 esters of glabretal (1). Oxidation of the mixture afforded a crystalline γ-lactone, m.p. 200—202°, again as a mixture of esters. The interrelation of the esters (3)—(5) with glabretal (1) was achieved in the following manner. The γ-lactone mixture was used since it avoided the further complication of the epimeric hemiacetals.

Osmylation of the γ-lactone mixture yielded two diols. The less polar diol (11), m.p. 218—220°, has a molecular ion at *m/e* 644 ($C_{37}H_{56}O_9$) in its mass spectrum with no trace of any lower homologues. The n.m.r. spectrum shows a new >C*H*OH resonance [δ 4·18 (q, *J* 7 Hz)] coupled to a methyl group. The C-7 acetate methyl signal appears as two singlets (δ 1·99 and 2·02), confirming that this is the expected mixture of diastereoisomers. The more polar diol (12), m.p. 203—205°, arises from the methacrylate (5) and has a parent ion at *m/e* 630 ($C_{36}H_{54}O_9$). In the n.m.r. it has signals for a primary hydroxy-group [δ 3·6 (q, *J* 9 Hz)] and two acetate methyl groups (δ 1·94 and 2·0), again reflecting the diastereoisomeric mixture.

Sodium metaperiodate cleavage of both diols (11) and

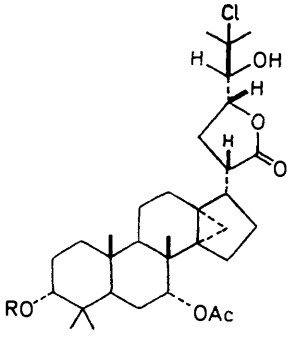
[7] C. Djerassi, O. Halpern, V. Halpern, and B. Riniker, *J. Amer. Chem. Soc.*, 1958, **80**, 4001.

(12) gave the same product, the pyruvate (13), $C_{35}H_{50}O_8$, m.p. 180—182° [δ 2·39 (s, Me·CO·CO)]. This result provides chemical proof that the original crystalline material is a mixture of C_4 and C_5 αβ-unsaturated esters which all have a methyl group attached to the α-carbon atom. The presence of angelate, tiglate, and methacrylate esters was confirmed by examination of the vinyl region of the n.m.r. spectrum of the original mixture of hemiacetals and the corresponding mixture of γ-lactones. A broad multiplet at δ 6·85 and a multiplet at 6·11 are characteristic of tiglate and angelate vinyl protons respectively, while the *exo*-methylene protons of the methacrylate appear at δ 5·52 and 6·11. The integration of these signals suggested that approximately equal amounts of each ester were present.

Sarrett oxidation of the two diols (11) and (12) also yielded the pyruvate (13) as the major product in each case. It is possible that this oxidation proceeds *via* a cyclic chromate ester. A minor product of the oxidation in each case was the keto-γ-lactone (15) which presumably arises by hydrolysis of the pyruvate ester followed by oxidation of the newly formed hydroxy-group.

Hydrolysis of the pyruvate (13) with aqueous sodium hydrogen carbonate furnished the hydroxy-γ-lactone (9), previously obtained from (2). Oxidation to the known keto-γ-lactone (15) again confirmed its identity. This series of reactions completes the interrelation of the first six compounds from the extract.

The hydroxy-γ-lactone (9) was used for the preparation



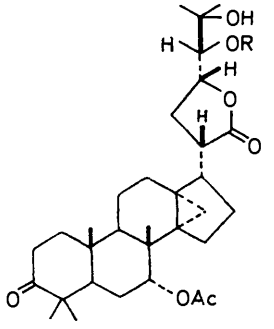
(18) R=CH_2Cl·CO–

(19) R=CH_2I·CO–

of suitable derivatives for *X*-ray analysis. Treatment of (9) with an excess of chloroacetyl chloride gave the chloracetate (18), m.p. 213—216°, with a chlorohydrin in the side chain. The corresponding iodoacetate (19), m.p. 184—185°, was obtained by reaction of (18) with potassium iodide in refluxing acetone.

Crystals of (18) and (19) are isomorphous and brief crystallographic details of the determination of their structures and relative stereochemistry, as in (18) and (19) and Figure 1, are given in the Experimental section. Crystal decomposition during *X*-ray data collection precluded determination of the absolute stereochemistry by Bijvoet's method.[8] However, the positive Cotton effect of the ketone (14) clearly demonstrates[9] the validity of the absolute configuration shown.

In other unsuccessful attempts to prepare a suitable *X*-ray derivative, the keto-γ-lactone (15) was refluxed with chloroacetic acid in benzene. This reaction afforded

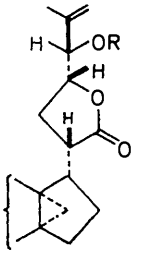


(20) R=CH_2Cl·CO–

(21) R=CH_2I·CO–

(22) R=CH_2Br·CO–

(23) R=H



(24) R=H

(25) R=Ac

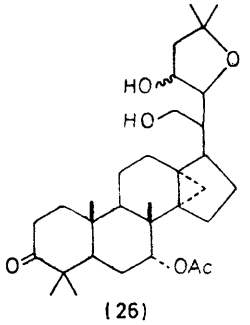
the chloroacetate (20), m.p. 195—196°, by apparent anti-Markownikoff opening (ester exchange?) of the epoxide [δ 4·90 (d, *J* 4 Hz, H-24), 4·06 (2H, s, $ClCH_2CO_2$–)]. The corresponding iodoacetate (21), m.p. 173—174°, and the related bromoacetate (22), m.p. 203—204°, prepared from (15) by reaction with bromoacetic acid, failed to provide satisfactory crystallographic data. Other products from the chloroacetic acid reaction included the hydroxy-olefin (24), m.p. 157—160° [δ 3·97 (d, *J* 7 Hz, >C*H*OH, H-24), 1·77br (s, vinyl methyl), and 5·03 (2H, m, *exo*-methylene)] and the diol (23), m.p. 198—200°. Acetylation of the former yielded the acetate (25), m.p. 110—115, 184°, which crystallised with a molecule of chloroform of crystallisation.

The most polar compound from the extract is assigned the tentative structure (26) on the following basis. It has the molecular formula $C_{32}H_{50}O_6$, and from spectroscopic evidence has a secondary acetate, a cyclohexanone, two hydroxy-functions {one primary and one secondary [δ 3·68 (2H, m) and 3·80 (1H, m)]}, and a cyclic ether [δ 3·38 (1H, m)]. In addition, six tertiary methyl signals can be observed. The characteristic resonances for the side chain hemiacetal and epoxide are absent. Oxidation of (26) afforded the γ-lactone (27), m.p. 174—178°, which lacks hydroxy-absorption in the i.r. The n.m.r. spectrum of (27) clearly shows cyclopropyl methylene protons at δ 0·36 and 0·6 (both d, *J* 5·5 Hz). In addition there are two mutually coupled signals at δ 3·98 (t, *J* 8 Hz, H-22) and 4·36 (m, $W_{\frac{1}{2}}$ 26 Hz, H-23). Irradiation at δ 3·9 caused the multiplet at δ 4·36 to collapse to a quartet

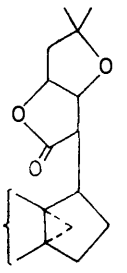
[8] J. M. Bijvoet, *Proc. k. ned. Akad. Wetenschap.*, 1949, **52**, 313.

[9] P. Crabbé, in 'Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry,' Holden-Day, San Francisco, 1965, pp. 44—45.

while the reverse experiment caused the resonance at δ 3·98 to become a doublet. Both protons were further coupled to protons in the δ 2·5 region. On the assumption that the carbon skeleton of (26) is the same as that of the other *Guarea* compounds the structure (27) can be



(26)



(27)

drawn for the γ-lactone. This, in turn, leads to the structure (26) for the original compound.

These *Guarea* compounds represent a novel and rather puzzling stage in the biogenetic scheme [4] for the triterpenoid metabolites of the Meliaceae. They could mark an intermediate step in the migration of a methyl group from C-14 to -13 during a dammarane–tirucallane rearrangement. This, however, seems unlikely in view of the presence of the 7-oxygen substituent. Insertion of a C-7 oxygen function in limonoids and related compounds has been considered to occur during the transformation of the tirucallane into the apotirucallane system. It is therefore more reasonable to assume that the glabretal carbon skeleton arises by capture of the 13-methyl group by a C-14 cation in an apotirucallane precursor.

EXPERIMENTAL

For general experimental details see Part I.[10]

Extraction.—The powdered heartwood of *G. glabra* (5 kg) was extracted with ethyl acetate in a Soxhlet apparatus for 48 h. The solvent was removed and the residue extracted into chloroform. The chloroform-soluble extract (70 g) was chromatographed over acidic alumina (Grade III, 2 kg). Elution with chloroform–light petroleum (1 : 3) furnished non-polar material (8 g) which on rechromatography gave β-sitostenone (1·5 g), m.p. 92—94° (needles from methanol) (Found: C, 84·0; H, 11·75. Calc. for $C_{29}H_{48}O$: C, 84·4; H, 11·7%), and β-sitosterol (3 g), m.p. 134—136°. Continued elution with the same solvents, gradually increasing the polarity, yielded the following compounds which were purified by a combination of preparative t.l.c. and crystallisation. The early fractions contained the mixture of esters (3)—(5) (10 g), which was crystallised from ethyl acetate–light petroleum, m.p. 137—139°, $\nu_{max.}$ (CCl_4) 3611, 1732, and 1715 cm^{-1}: δ 5·51 (m, H-21), 4·97 (m, H-7), 4·65 (m, H-3), 3·77 (m, H-23), 2·84 and 2·67 (both d, *J* 7 Hz, H-24, epimeric mixture); *m/e* 612 and 518. The *ketone* (14) (6 g) was obtained next, after crystallisation from ether–light petroleum had m.p. 172—175°, $[\alpha]_D$ −4° (*c* 1·0), $\nu_{max.}$ (CCl_4) 3610, 1735, and 1709 cm^{-1}; δ 5·25 (m, H-21), 4·96 (m, H-7), 3·75 (m, H-23), 3·34 (d, *J* 7 Hz, H-24), 1·92 (acetate), and 1·21 (6H), 1·11, 0·96, and 0·93 (6H) (tertiary methyls) (Found: C, 72·8; H, 9·15. $C_{30}H_{48}O_6$ requires C, 72·7; H, 9·40%). Further elution gave the *2-hydroxy-3-methylbutyrate* (2), (7 g), which crystallised from ethyl acetate–light petroleum as needles, m.p. 118—130, 198—200°, $[\alpha]_D$ −49° (*c* 1·5), $\nu_{max.}$ (CCl_4) 3610, 3540, 3430, and 1730 cm^{-1}; δ 5·36 (m, H-21), 4·95 (m, H-7), 4·71 (m, H-3), 2·85 and 2·68 (both d, *J* 7 Hz, H-24, epimeric mixture), and 3·85 and 3·02 (disappeared on addition of D_2O, OH) (Found: C, 70·4; H, 9·35. $C_{37}H_{58}O_8$ requires C, 70·45; H, 9·25%). *Glabretal* [(20S,23R)-*21,23:24,25-diepoxy-3α,21-dihydroxy-4,4,8β-trimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-7α-yl acetate*] (1) (3·5 g), was obtained as an oil (*m/e* 530) (Found: C, 72·55; H, 9·35. $C_{32}H_{50}O_6$ requires C, 72·4; H, 9·5%). The most polar compound, the *diol* (26) (30 mg), $\nu_{max.}$ (CCl_4) 3630, 3610, 3450, 1738, and 1712 cm^{-1}; δ 4·98 (m, H-7), 1·23 (6H), and 1·08 and 0·97 (9H) (tertiary methyls), was eluted with ethyl acetate and could not be crystallised: it was characterised as the γ-lactone (27).

Oxidation of Glabretal (1).—Glabretal (1) (48 mg) in dry pyridine (10 ml) was treated with an excess of chromium trioxide (80 mg) and the reaction left for 12 h. The crude product was purified by preparative t.l.c. and then crystallisation from ethyl acetate–light petroleum to give the *keto-γ-lactone* (15) (36 mg), as needles, m.p. 215—218°, $[\alpha]_D$ −35° (*c* 1·5), δ 5·07 (m, H-7), 4·10 (m, H-23), 2·80 (d, *J* 7 Hz, H-24), 2·05 (acetate), 1·37 (6H), 1·16, and 1·03 (9H) (tertiary methyls), and 0·66 and 0·34 (ABq, *J* 6 Hz, cyclopropyl methylene) (Found: C, 73·1; H, 8·85. $C_{32}H_{46}O_6$ requires C, 73·0; H, 8·8%).

Reduction of the Keto-γ-lactone (15).—The keto-γ-lactone (15) (70 mg) in absolute alcohol was treated with an excess of sodium borohydride at room temperature for 17 h. Preparative t.l.c. of the product afforded the oily *triol* (17) (52 mg), $[\alpha]_D$ −5° (*c* 1·8), δ 4·99 (m, H-7), 3·1—3·8 (4H, *CH*OH and *CH*$_2$OH), 3·74 (d, *J* 9 Hz, H-24), and 2·01 (acetate) (Found: C, 72·05; H, 9·95. $C_{32}H_{52}O_6$ requires C, 72·15; H, 9·85%).

The Acetoxy-γ-lactone (6).—Glabretal (1) (105 mg) was treated with acetic anhydride (10 ml) in pyridine (10 ml) for 12 h. The resultant diacetate was adsorbed on basic alumina (10 g) in benzene for 24 h. The alumina was washed with chloroform and preparative t.l.c. of the product then gave a monoacetate (50 mg). Oxidation under the normal Sarrett conditions afforded the *acetoxy-γ-lactone* (6) (35 mg) which crystallised from ethyl acetate–light petroleum as needles, m.p. 220—222°, $[\alpha]_D$ −69° (*c* 1·6), $\nu_{max.}$ (CCl_4) 1783 and 1732 cm^{-1} (Found: C, 71·55; H, 8·7. $C_{34}H_{48}O_7$ requires C, 71·8; H, 8·5%).

Oxidation of the Ketone (14).—The ketone (14) (63 mg) was oxidised under normal Sarrett conditions to give the keto-γ-lactone (15) (49 mg) which crystallised from ethyl acetate–light petroleum as needles, m.p. 210—213°, identical with the oxidation product of glabretal (1).

The Acetoxy-γ-lactone (7).—The ester (2) (92 mg) was acetylated at room temperature for two days in acetic anhydride–pyridine. The resulting diacetate was treated with basic alumina in benzene for 48 h. Preparative t.l.c. of the product afforded the *hydroxy-acetate* (44 mg) which crystallised from ethyl acetate–light petroleum as needles, m.p. 185—187°, $[\alpha]_D$ −40° (*c* 1·0), $\nu_{max.}$ (CCl_4) 3615, 1742, and 1735 cm^{-1} (Found: C, 69·4; H, 9·05. $C_{39}H_{60}O_9$ requires C, 69·6; H, 9·0%). Sarrett oxidation and crystallisation from ethyl

[10] J. D. Connolly, R. Henderson, R. McCrindle, K. H. Overton, and N. S. Bhacca, *J. Chem. Soc.*, 1965, 6935.

acetate–light petroleum furnished the *acetoxy-γ-lactone* (7) (37 mg), as needles, m.p. 204—215°, $\nu_{max.}$ (CCl_4) 1782 and 1737 cm^{-1}; δ 4·99 (m, H-7), 4·91 (d, *J* 4 Hz, >C*H*OAc), 4·70 (m, H-3), 2·77 (d, *J* 7 Hz, H-24), and 2·14 and 2·02 (acetates) (Found: C, 70·05; H, 8·75. $C_{39}H_{58}O_9$ requires C, 69·85; H, 8·7%).

Oxidation of the Ester (2).—The ester (2) (141 mg) was oxidised under the usual Sarrett conditions. The product was separated into two components by preparative t.l.c. The less polar compound, the *α-keto-ester* (8) (82 mg), was obtained as needles from ethyl acetate–light petroleum, m.p. 209—210°, $\nu_{max.}$ ($CHCl_3$) 1768 and 1720 cm^{-1}; δ 4·98 (m, H-7), 4·83 (m, H-3), 4·13 (m, H-23), 3·18 (septet, *J* 7 Hz, Me_2CH), 2·67 (d, *J* 8 Hz, H-24), and 2·0 (acetate) (Found: C, 71·15; H, 8·7. $C_{37}H_{54}O_8$ requires C, 70·9; H, 8·7%). The more polar constituent, the *α-hydroxy-ester* (10) (52 mg) crystallised from ethyl acetate–light petroleum as fine needles, m.p. 225—226°, $\nu_{max.}$ (CCl_4) 3535, 1779, and 1724 cm^{-1}; δ 5·0 (m, H-7), 4·76 (m, H-3), 2·77 (d, *J* 6 Hz, H-24), and 1·99 (acetate) (Found: C, 70·5; H, 9·1. $C_{37}H_{56}O_8$ requires C, 70·65; H, 9·0%).

Hydrolysis of the α-Keto-ester (8).—The α-keto-ester (8) (60 mg) in methanol (30 ml) was stirred for 15 h with a saturated aqueous solution of sodium hydrogen carbonate. Water was added and the solution extracted with chloroform. Preparative t.l.c. and crystallisation from ethyl acetate–light petroleum yielded the *hydroxy-γ-lactone* (9) (50 mg) as plates, m.p. 202—204°, $\nu_{max.}$ (CCl_4) 3630, 1779, and 1728 cm^{-1}; δ 4·98 (m, H-7), 4·10 (m, H-23), 3·38 (m, H-3), 2·74 (d, *J* 7 Hz, H-24), and 1·99 (acetate) (Found: C, 70·35; H, 9·2. $C_{32}H_{48}O_6,H_2O$ requires C, 70·3; H, 9·2%). Sarrett oxidation of (9) afforded the known keto-γ-lactone (15), m.p. 215—218°.

Oxidation of the Ester Mixture (3)—(5).—The mixture (425 mg) was treated with an excess of chromium trioxide in pyridine (5 ml) for 3 days. Work-up and crystallisation from ethyl acetate–light petroleum gave the γ-lactone mixture (410 mg), m.p. 200—202°.

Hydroxylation of the γ-Lactone Mixture.—The γ-lactone mixture (400 mg) in dry diethyl ether (20 ml) and pyridine (5 ml) was treated with osmium tetraoxide (500 mg) at room temperature for 24 h. Hydrogen sulphide gas was passed briefly through the solution which was then filtered through Celite. The product consisted of two compounds of similar polarity which were separated by repeated preparative t.l.c. The less polar *diol* (11) (155 mg) crystallised from ethyl acetate–light petroleum as needles, m.p. 218—220°, $\nu_{max.}$ (CCl_4) 3584, 3515, 1780, and 1732 cm^{-1}; δ 5·02 (m, H-7), 4·76 (m, H-3), 3·86 (m, H-23), 2·8 (d, *J* 7 Hz, H-24), and 2·02 and 1·99 (diastereoisomeric acetates) (Found: C, 68·8; H, 8·75. $C_{37}H_{56}O_9$ requires C, 68·8; H, 8·75%). The more polar *diol* (12) (160 mg) also crystallised from ethyl acetate–light petroleum as fine needles, m.p. 203—205°, $\nu_{max.}$ (CCl_4) 3585, 3520, 1785, and 1737 cm^{-1}; δ 4·99 (m, H-7), 4·71 (m, H-3), 4·10 (m, H-23), 2·75 (d, *J* 7 Hz, H-24), and 2·0 and 1·96 (diastereoisomeric acetates) (Found: C, 66·7; H, 8·5. $C_{36}H_{54}O_9,H_2O$ requires C, 66·65; H, 8·7%).

Sodium Metaperiodate Oxidation of the Diols (11) *and* (12).—The diol (11) (40 mg) in methanol (1 ml) was stirred overnight with a saturated aqueous solution of sodium metaperiodate. Work-up in the usual manner followed by crystallisation from ethyl acetate–light petroleum afforded the *pyruvate* (13) (30 mg) as fine needles, m.p. 189—192°, $\nu_{max.}$ (CCl_4) 1784, 1730, and 1724 cm^{-1}; δ 4·97 (m, H-7), 4·74 (m, H-3), 4·12 (m, H-23), 2·73 (d, *J* 8 Hz, m-24), and 1·99 (acetate) (Found: C, 68·45; H, 8·3. $C_{35}H_{50}O_8,H_2O$ requires C, 68·15; H, 8·5%).

The diol (12) also gave the pyruvate (13), m.p. 189—192°, on reaction with sodium metaperiodate.

Sarrett Oxidation of the Diols (11) *and* (12).—Oxidation of the diol (11) (30 mg) under the usual Sarrett conditions gave a mixture of two products which were separated by preparative t.l.c. The less polar product was the pyruvate (13) (19 mg), m.p. 179—181°, and the more polar, minor, component was the keto-γ-lactone (15) (4 mg), m.p. 213—215°.

Oxidation of the diol (12) under these conditions afforded the same two products.

Hydrolysis of the Pyruvate (13).—The pyruvate (13) (82 mg) in methanol (50 ml) was stirred for 4 h with a saturated aqueous solution of sodium hydrogen carbonate. The crude product was purified by preparative t.l.c. and crystallisation from ethyl acetate–light petroleum to give the hydroxy-γ-lactone (9), m.p. 202—204°, identical with the compound obtained by hydrolysis of the α-keto-ester (8). Sarrett oxidation of (9) gave the known keto-γ-lactone (15), m.p. 215—218°.

The Chloroacetate (18) *and Iodoacetate* (19).—The hydroxy-γ-lactone (9) (103 mg) was treated with an excess of chloroacetyl chloride at room temperature for 3 h. Preparative t.l.c. and crystallisation from ethyl acetate–light petroleum afforded the chloroacetate (18) (92 mg), m.p. 213—216°, $\nu_{max.}$ (CCl_4) 3580, 1775, and 1730 cm^{-1}; δ 4·98 (m, H-7), 4·84 (t, $J_{obs.}$ 8 Hz, H-23), 4·74 (m, H-3), 4·04 ($CH_2Cl{\cdot}CO_2O$–), 3·58br (s, H-24), 1·99 (acetate), 1·64 and 1·56 (tertiary methyls, C-26 and -27), and 1·07, 0·89, 0·86, and 0·76 (tertiary methyls). The chloroacetate (18) was heated in refluxing acetone with an excess of potassium iodide for 4 h. The crude product was crystallised from ethyl acetate–light petroleum to give the *iodoacetate* (19), m.p. 184—185°, δ 4·98 (m, H-7), 4·84 (t, $J_{obs.}$ 8 Hz, H-23), 4·65 (m, H-3), 3·69 ($CH_2I{\cdot}CO_2$–), 3·54br (s, H-24), 2·02 (acetate), 1·64 and 1·55 (tertiary methyls, C-26 and -27), and 1·07, 0·89, 0·84, and 0·80 (tertiary methyls) (Found: C, 55·5; H, 6·75. $C_{34}H_{50}ClIO_7$ requires C, 55·7; H, 6·9%).

Reaction of (15) *with Chloroacetic Acid.*—The keto-γ-lactone (15) (207 mg) in anhydrous benzene was refluxed with chloroacetic acid (200 mg) for 4 h. The solution was filtered through a short column of neutral alumina to give a mixture of three components which were separated by preparative t.l.c. The least polar compound was the *hydroxy-olefin* (24) (68 mg) which crystallised from ethyl acetate–light petroleum, m.p. 157—160°, $\nu_{max.}$ (CCl_4) 3588, 3445, 1780, 1734, and 1705 cm^{-1}; δ 5·03 (m, *exo*-methylene and H-7), 3·97 (d, *J* 7 Hz, H-24), and 2·01 (acetate) (Found: C, 73·2; H, 8·7. $C_{32}H_{46}O_6$ requires C, 72·95; H, 8·8%). The second compound, the *chloroacetate* (20) (79 mg), also crystallised from ethyl acetate–light petroleum as needles, m.p. 195—196°, $\nu_{max.}$ (CCl_4) 3590, 3480, 1780, 1735, and 1708 cm^{-1}; δ 5·0 (m, H-7) and 2·02 (acetate) (Found: C, 65·8; H, 7·95. $C_{34}H_{49}ClO_8$ requires C, 65·75; H, 7·9%). The most polar compound, the *diol* (23) (18 mg) was also crystallised from ethyl acetate–light petroleum, m.p. 198—200°, $\nu_{max.}$ (CCl_4) 3565, 3520, 1782, 1723, and 1707 cm^{-1} (Found: C, 67·4; H, 8·85. $C_{32}H_{48}O_7,\frac{3}{2}H_2O$ requires C, 67·25; H, 8·95%).

The Iodoacetate (21).—The chloroacetate (20) (75 mg) was heated in refluxing acetone for 15 h with an excess of sodium iodide. The resulting *iodoacetate* (21) (56 mg), after purification by t.l.c. and crystallisation from chloroform–light petroleum, formed needles, m.p. 173—174°, $\nu_{max.}$ (CCl_4) 3590,

1765, 1724, and 1700 cm^{-1}; δ 5·03 (m, H-7), 4·88 (d, J 3 Hz, H-24), 3·75 ($CH_2I{\cdot}CO_2$–), and 2·0 (acetate) (Found: C, 57·25; H, 7·05. $C_{34}H_{49}IO_8$ requires C, 57·3; H, 6·9%).

The Bromoacetate (22).—The keto-γ-lactone (15) (150 mg) was refluxed in benzene with bromoacetic acid for 15 h. The major product, the *bromoacetate* (22) (64 mg) was isolated by preparative t.l.c. and crystallised from chloroform–light petroleum as needles, m.p. 201—202°, $\nu_{max.}$ (CCl_4) 3590, 1765, 1724, and 1700 cm^{-1}; δ 5·02 (m, H-7), 4·88 (d, J 3 Hz, H-24), 4·71 (m, H-23), 3·85 ($CH_2Br{\cdot}CO_2$–), and 1·98 (acetate) (Found: C, 61·3; H, 7·35. $C_{34}H_{49}BrO_8$ requires C, 61·35; H, 7·35%).

Acetylation of the Hydroxy-olefin (24).—The hydroxy-olefin (24) (26 mg) was treated at room temperature with acetic anhydride in pyridine for 48 h. The *acetate* (25) was purified by preparative t.l.c. and crystallised from chloroform–light petroleum as large needles, m.p. 110—115, 184° (Found: C, 61·0; H, 7·25. $C_{34}H_{48}O_7{,}CHCl_3$ requires C, 61·1; H, 7·1%).

The γ-Lactone (27).—Compound (26) (14 mg) in dry pyridine was treated with chromium trioxide (15 mg) for 15 h. Preparative t.l.c. and crystallisation from ethyl acetate–light petroleum afforded the *γ-lactone* (27) (10 mg), m.p. 174—178°, $[\alpha]_D$ −24° (*c* 0·7), $\nu_{max.}$ (CCl_4) 1790, 1737, and 1710 cm^{-1}; δ 5·0 (m, H-7), 2·0 (acetate), and 1·10 and 0·98 (15H) (tertiary methyls) (Found: M^+, 526·3288. $C_{32}H_{46}O_6$ requires M, 526·3294).

X-*Ray Analysis*.—The derivatives (18) and (19) were recrystallised from ethyl acetate–light petroleum as small plates and were subsequently shown to be isostructural.

Crystal Data.—Chloroacetate (18), $C_{34}H_{50}O_7Cl_2$, M = 641·7. Monoclinic, a = 16·217(3), b = 7·321(2), c = 15·990(2) Å, β = 115·35(2)°, U = 1716 Å^3, Z = 2, D_c = 1·24 g cm^{-3}, $F(000)$ = 684. Space group $P2_1$. Cu-K_α radiation, λ = 1·5418 Å, μ(Cu-K_α) = 20·7 cm^{-1}.

Iodoacetate (19), $C_{34}H_{50}ClIO_7$, M = 733·1. Monoclinic, a = 16·736(2), b = 7·262(2), c = 16·127(5) Å, β = 115·54(2), U = 1769 Å^3, Z = 2, D_c = 1·38 g cm^{-3}, $F(000)$ = 758. Space group $P2_1$. Cu-K_α radiation, λ = 1·5418 Å, μ(Cu-K_α) = 83·3 cm^{-1}.

For both derivatives the space group symmetry and initial unit cell parameters were obtained from rotation, Weissenberg, and precession photographs taken with Cu-K_α radiation. Both crystals had approximate dimensions 0·1 × 0·2 × 0·3 mm^3. The only systematic absences are $0k0$ absent if k is odd which, taken with the optically active nature of the compounds, uniquely determines the space group as $P2_1$.

For both compounds accurate parameters were obtained by a least-squares treatment of the setting angles of 12 reflections measured on a Hilger and Watts Y290 computer-controlled diffractometer. Three-dimensional intensity data were collected to a maximum Bragg angle (Cu-K_α) of 50°, as preliminary photographic investigations had shown that intensities fell off fairly rapidly with increasing θ. The θ—2θ scan technique was used with Ni-filtered Cu-radiation and a symmetric scan of 80 steps of 0·01° with a second count at each step. Stationary crystal–stationary counter background counts of 20 s duration were measured at each end of the integrated scan. Three standard reflections were monitored at approximately hourly intervals throughout both data collections. There was considerable decomposition of the crystal of (19) as judged by the variation of the standard reflections (33·5% drop) but data for (18) seemed much less affected by exposure to radiation (4·7% variation in standards).

Each intensity was corrected for background and the estimated standard deviation for each intensity σ(I) was calculated from the expression $\sigma(I) = [S + 4B + (0{\cdot}05S)^2]^{\frac{1}{2}}$ where S and B are the scan and sum of background counts, respectively. In all, 1040 intensities $>3\sigma(I)$ for (19) and 1539 data with intensities $>2\sigma(I)$ for (18) were obtained. The data were corrected for Lorentz and polarisation factors and for the effect of decomposition, assumed linear between standards. All calculations were carried out on the IBM 370/55 computer with locally modified programs for data-handling and the X-ray 72 system.[11]

TABLE 1

Fractional co-ordinates, with estimated standard deviations in parentheses, for the chloroacetate (18)

Atom	x	y	z
Cl(1)	0·8408(4)	0·7411 *	−0·2198(4)
Cl(2)	1·2702(6)	1·2540(13)	0·5222(6)
O(3)	0·6640(6)	0·7420(16)	−0·2099(6)
O(7)	0·8406(6)	0·3411(14)	0·0140(6)
O(20)	1·0849(10)	0·5550(20)	0·5581(8)
O(22)	1·1677(7)	0·7606(17)	0·5271(6)
O(23)	1·2836(10)	0·7517(27)	0·4456(12)
O(29)	0·8543(11)	0·0515(21)	−0·0238(14)
O(31)	0·6036(10)	0·9309(27)	−0·3279(9)
C(1)	0·6271(9)	0·8106(22)	−0·0510(9)
C(2)	0·5553(9)	0·8205(23)	−0·1500(9)
C(3)	0·5746(9)	0·6892(21)	−0·2138(9)
C(4)	0·5867(9)	0·4891(20)	−0·1815(9)
C(5)	0·6599(9)	0·4838(20)	−0·0799(8)
C(6)	0·6798(8)	0·2914(20)	−0·0397(8)
C(7)	0·7717(7)	0·2856(18)	0·0461(7)
C(8)	0·7743(8)	0·4226(19)	0·1212(8)
C(9)	0·7341(8)	0·6075(20)	0·0791(8)
C(10)	0·6413(8)	0·6107(20)	−0·0101(8)
C(11)	0·7325(9)	0·7395(23)	0·1562(9)
C(12)	0·8242(11)	0·7620(30)	0·2436(11)
C(13)	0·8921(10)	0·6072(24)	0·2558(10)
C(14)	0·8741(8)	0·4533(19)	0·1942(8)
C(15)	0·9287(9)	0·2878(23)	0·2531(9)
C(16)	1·0043(10)	0·3804(26)	0·3449(11)
C(17)	0·9555(9)	0·5638(23)	0·3536(10)
C(18)	1·0222(10)	0·7194(24)	0·4082(9)
C(19)	0·5561(9)	0·5605(21)	0·0074(10)
C(20)	1·0914(13)	0·6564(23)	0·5046(13)
C(21)	1·0835(10)	0·8065(26)	0·3658(10)
C(22)	1·1577(10)	0·8982(24)	0·4523(10)
C(23)	1·2470(13)	0·9167(35)	0·4513(11)
C(24)	1·3182(12)	1·0437(30)	0·5274(11)
C(25)	0·9357(10)	0·5889(24)	0·1768(10)
C(26)	0·4904(10)	0·4093(24)	−0·2006(11)
C(27)	0·6239(12)	0·3818(25)	−0·2419(11)
C(28)	0·7198(11)	0·3293(21)	0·1688(12)
C(29)	0·8754(10)	0·2153(26)	−0·0205(12)
C(30)	0·9462(12)	0·2923(32)	−0·0444(14)
C(31)	0·6671(11)	0·8524(23)	−0·2712(10)
C(32)	0·7599(13)	0·9076(26)	−0·2581(14)
C(33)	1·3506(10)	0·9497(34)	0·6293(10)
C(34)	1·4036(14)	1·0656(43)	0·5037(16)

* This position has been held constant to fix the origin of the unit cell.

Structure Determination.—Initially only crystals of (19) were available and the poor quality of the data obtained prompted us to prepare other derivatives while attempting to solve the structure. A three dimensional Patterson function yielded co-ordinates of the iodine atom and despite pseudo-symmetry problems in the early Fourier syntheses, the bulk of the structure of (19) soon became apparent. However, all attempts at refinement could not lower $R = \Sigma(||F_o| - |F_c||)/\Sigma|F_o|$ below 0·26.

[11] Technical Report, TR-192, Computer Science Centre, University of Maryland, June 1972.

At this time good quality data for (18) became available and by using the ' final ' co-ordinates from the structure of (19) as the starting set for a round of structure factor and

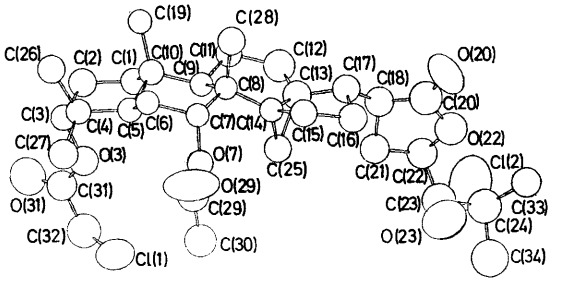
TABLE 2

Torsional angles (degrees) * for the chloroacetate (18)

C(1)–C(2)–C(3)–C(4)	55	C(17)–C(13)–C(14)–C(15)	0·2
C(2)–C(3)–C(4)–C(5)	−53	C(13)–C(14)–C(15)–C(16)	−19
C(3)–C(4)–C(5)–C(10)	55	C(14)–C(15)–C(16)–C(17)	29
C(4)–C(5)–C(10)–C(1)	−55	C(15)–C(16)–C(17)–C(13)	−28
C(5)–C(10)–C(1)–C(2)	53	C(16)–C(17)–C(13)–C(14)	19
C(10)–C(1)–C(2)–C(3)	−55		
		C(20)–C(18)–C(21)–C(22)	−37
C(10)–C(5)–C(6)–C(7)	−69	C(18)–C(21)–C(22)–O(22)	36
C(5)–C(6)–C(7)–C(8)	57	C(21)–C(22)–O(22)–C(20)	−24
C(6)–C(7)–C(8)–C(9)	−44	C(22)–O(22)–C(20)–C(18)	0·5
C(7)–C(8)–C(9)–C(10)	46	O(22)–C(20)–C(18)–C(21)	23
C(8)–C(9)–C(10)–C(5)	−55		
C(9)–C(10)–C(5)–C(6)	63	C(8)–C(14)–C(25)–C(13)	111
		C(14)–C(25)–C(13)–C(12)	−115
C(11)–C(9)–C(8)–C(14)	−62	C(25)–C(13)–C(12)–C(11)	67
C(9)–C(8)–C(14)–C(13)	43		
C(8)–C(14)–C(13)–C(12)	−12	C(13)–C(25)–C(14)–C(15)	−101
C(14)–C(13)–C(12)–C(11)	−1	C(25)–C(14)–C(15)–C(16)	56
C(13)–C(12)–C(11)–C(9)	−19	C(17)–C(13)–C(25)–C(14)	102
C(12)–C(11)–C(9)–C(8)	52		
		C(13)–C(17)–C(18)–C(20)	−174
		C(13)–C(17)–C(18)–C(21)	−54
		C(16)–C(17)–C(18)–C(20)	−54
		C(16)–C(17)–C(18)–C(21)	66

* The estimated standard deviations are in the range 0·9—2·3°.

electron density calculations we quickly obtained a satisfactory set of parameters for (18). Refinement with unit weights, initially with isotropic but finally with anisotropic thermal parameters, lowered R to its final value of 0·109 and clearly established the molecular structure as (18). During the final stages of refinement, a weighting scheme such that $W^{\frac{1}{2}} = [\sigma^2(F) + 2{\cdot}5 \times 10^{-3}F^2]^{-\frac{1}{2}}$ proved satisfactory. Work was stopped at this stage as our objective of determining the structure had been achieved and the computing budget for the structure exceeded. Bond length and angle calculations with the final co-ordinates showed no unusual features and intermolecular contacts are normal. All the various data tables for (18) are listed in Supplementary Publication No.



SUP 21221 (21 pp., 1 microfiche),* and include thermal parameters, bond lengths and angles, and *hkl*, F_o and F_c values. Fractional co-ordinates and torsion angles are listed in Tables 1 and 2 and an ORTEP diagram [12] of the molecular structure (18), including the atomic numbering system, is shown in the Figure.[12] No data are given for (19) because a satisfactory refinement could not be achieved.

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* For details of Supplementary Publications, see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1973, Index issue.

[12] C. K. Johnson, ORTEP 1965, Technical Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tennessee.