579. *Marrubiin. Part II.*[1] *Correlation with Ambreinolide.*

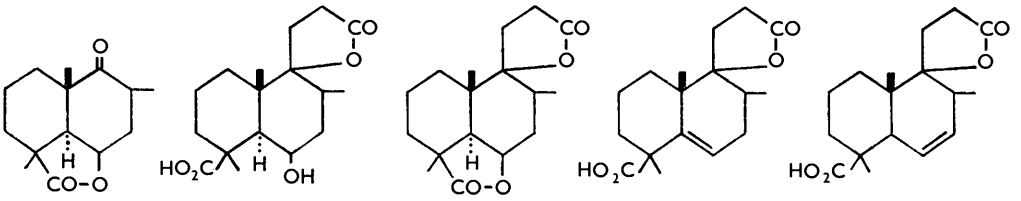
By D. Burn and W. Rigby.

The ring system of marrubiin is shown to be substituted in such a way that aromatisation is impossible without group migration. That the structure of the keto-lactone (I) is directly related to that of marrubiin is shown by preparing this lactone from marrubiin by a route in which the possibility of rearrangements' occurring is minimised : a new method of stepwise degradation of lactone rings is introduced. The skeleton of marrubiin is correlated with that of ambreinolide, and its stereochemistry is discussed.

The accepted formula of marrubiin is based on very little evidence, and it is difficult to reconcile the lack of reactivity of some of the degradation products with their proposed α-ketol and α-glycol structures. The 8-hydroxy-1 : 6 : 10-trimethyl-5-oxodecalin-1-carboxylic lactone (I) used in the investigation of the ring system [1] might itself have arisen from a molecular rearrangement. It was desirable that further checks should be made and that correlation should be established with substances of known structure. During the preparation of the starting materials, the isolation of marrubiin from white horehound

[1] Part I, Hardy and Rigby, preceding paper.

has been improved and some other constituents of the plant have been isolated. Conversion of the γ-lactone (II) into the dilactone (IIa) was best effected by simple heating: the acetic anhydride procedure of Cocker *et al.*[2] gave a mixture. It is unlikely that rearrangement occurs during the formation of these lactones from marrubiin, so they were used as the starting point for the investigations.



(I) (II) (IIa) (III) (IV)

Treatment of the lactonic hydroxy-acid (II) with toluene-*p*-sulphonyl chloride in pyridine gave the γ-lactone (III), the dilactone (IIa), and another substance which was not further investigated. That the unsaturated acid has structure (III) rather than the isomeric (IV) was shown by its smooth decarboxylation at the melting point, behaviour consistent with its formulation as a βγ-unsaturated acid. It was inert to catalytic hydrogenation, and various oxidation procedures gave only uncharacterised oils.

The product of thermal decarboxylation would be the lactone (V) which was readily hydrogenated to the saturated lactone (VI). Oxidation of the unsaturated lactone (V) by chromic acid or neutral or alkaline permanganate produced only oils, but potassium permanganate in acetic acid gave the epoxide (VII), and osmium tetroxide gave the dihydroxy-lactone (VIII).

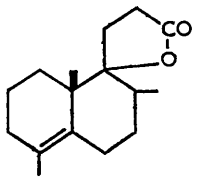
Selenium dioxide dehydrogenated the unsaturated lactone (V) to give, in poor yield, the diunsaturated lactone (IX) (or a $\Delta^{4:5}$-isomer); this heteroannular diene ($\lambda_{max.}$ 2300, 2370, 2440 Å) was also formed (in good yield) when the epoxide (VII) was boiled with aqueous-alcoholic sulphuric acid.

In order to test whether the ring system of marrubiin was such that either of the rings could be aromatised, introduction of a third double bond into the diene was attempted: easy aromatisation would imply absence of bridge-head alkyl groups or *gem.*-dimethyl groups in the ring concerned. When the diene was treated with sulphuric or phosphoric acid, oils resulted, but their ultraviolet absorption spectra were similar to that of the diene: if dehydration of the virtual hydroxyl group of the lactone ring had indeed occurred, the resulting double bond was not conjugated with the other two. As it was possible that its incorporation in the lactone ring was preventing the dehydration of the tertiary alcohol, dehydrating procedures were tried on the glycol (X) which resulted from reduction of the diene with lithium aluminium hydride. When this glycol was acted upon by phosphorus trichloride under conditions known to dehydrate marrubiin, no crystalline products could be isolated, but again the resulting oils showed only the original diene absorption: either the ring structure is such that aromatisation is impossible, or some conformational feature inhibits the necessary double-bond migrations. As it seemed likely (see below) that dehydration under alkaline conditions could result in the introduction of an endocyclic double bond, the diene-lactone (IX) was heated at 200° with sodium hydroxide in diethylene glycol. The resulting oil had an ultraviolet absorption maximum at 3120 Å and so presumably contained some of the expected triene. These findings are consistent with the formulæ used.

That no molecular rearrangement is involved when marrubiin is converted into the keto-lactone (I) was shown by preparing this substance by an alternative route. Treatment of the dilactone (IIa), resulting from the oxidation of marrubiin, with ethyl oxalate

[2] Cocker, Cross, Duff, Edward, and Holley, *J.*, 1953, 2540.

gave the ethoxalyl-dilactone (XI), which with sulphuryl chloride gave the α-chloro-α-ethoxalyl derivative (XII). This did not crystallise well but readily gave a crystalline hemiacetal with methyl alcohol; it was hydrolysed smoothly to the α-chloro-dilactone (XIII) which, however, proved difficult to degrade further.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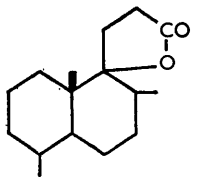
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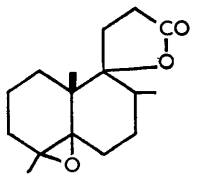
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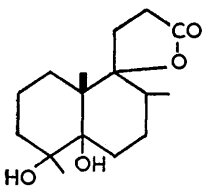
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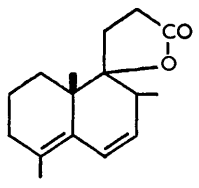
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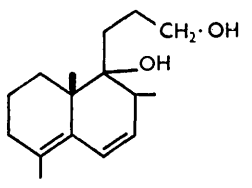
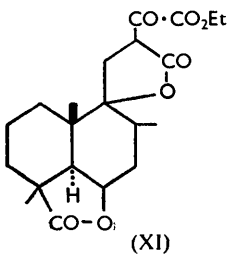

(X)

Ozonolysis of the ethoxalyl-dilactone (XI) yielded some of the expected α-keto-lactone (XIV), but the main product was a substance $C_{17}H_{22}O_6$, m. p. 195·5—197°. The α-keto-lactone had the expected spectral characteristics ($\lambda_{max.}$ 2340 Å, displaced to 2700 Å in alkali) and it gave a purple colour with ferric chloride, although under most conditions this colour reaction was obscured by the formation of an orange compound; it titrated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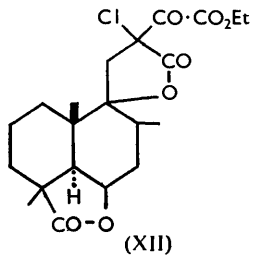
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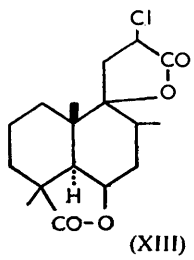
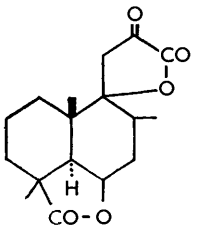

(XIII)

as a monobasic acid. When heated at 50—70° above its m. p., the α-keto-lactone lost 1 mol. of carbon dioxide and 1 mol. of carbon monoxide and gave the olefinic lactone (XV); the expected [3,4] aldehyde (XVI) was not found. The other product of the ozonolysis, the substance $C_{17}H_{22}O_6$, contained no alkoxyl, aldehyde, or ketone group; when hydroxylamine hydrochloride was added to its solution in pyridine, there was brisk evolution of carbon dioxide (1 mol.) and a hydroxamic acid was produced. It is thus the mixed carbonic anhydride (XVIII), and the hydroxamic acid is the lactone (XIX). Consistently with its formulation, the anhydride has infrared absorption maxima at 1827 and 1766 $cm.^{-1}$ (in potassium bromide). It may be supposed that the ozonolysis takes the courses shown in the annexed formulæ, the proposed route to the anhydride having some analogy with the formation of anhydrides when α-keto-esters react with per-acids.[5] When the mixed carbonic anhydride was melted (m. p. 197°), it lost carbon dioxide (*ca.* 1·5 mol.) and was converted into approximately equal proportions of the olefinic lactone (XV) and the related αβ-unsaturated acid (XVII); when the latter was heated a little above its m. p. it

[3] Plattner and Jampolsky, *Helv. Chim. Acta*, 1943, **26**, 687.
[4] Rossi and Schinz, *ibid.*, 1948, **31**, 473; Schinz and Rossi, *ibid.*, p. 1953.
[5] Karrer and Haab, *ibid.*, 1949, **32**, 950.

lost carbon dioxide (1 mol.) and gave the same olefinic lactone (XV) in low yield. Ozonolysis of the olefinic lactone gave formaldehyde and 8-hydroxy-1 : 6 : 10-trimethyl-5-oxodecalin-1-carboxylic lactone (I), identical with the product obtained by oxidation of anhydromarrubiin or anhydrotetrahydromarrubiin. Besides indicating that this C_{14}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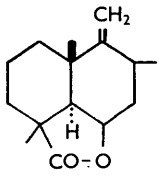
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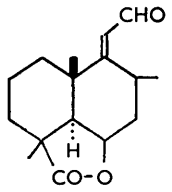
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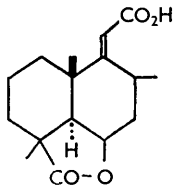
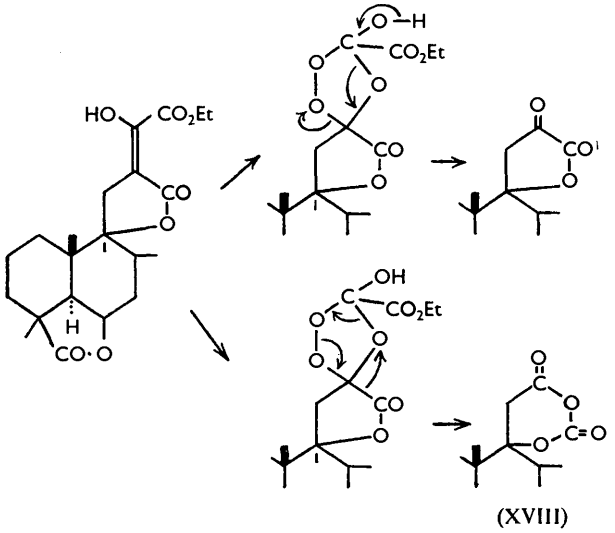

(XVII)

keto-lactone is not a rearrangement product, the scheme outlined serves to establish the structure of the degraded lactone ring as (XX) : the presence of two hydrogen atoms on the α-carbon atom follows from the possibility of forming the α-keto-lactone and the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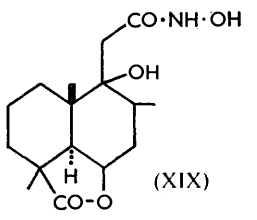
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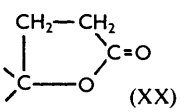
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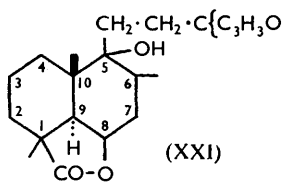

(XXI)

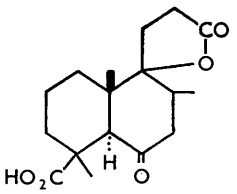
α-chloro-α-ethoxalyl-lactone; and the presence of two hydrogen atoms on the β-carbon atom follows from the formation of formaldehyde in the final ozonolysis.

The procedure described seems to be a general one for the stepwise degradation of such lactone rings : the variation involving pyrolysis of the carbonic anhydride is more satisfactory than that going by the keto-lactone, the temperature required being much lower and the products cleaner. The partial skeleton (XXI) for marrubiin is thus deduced.

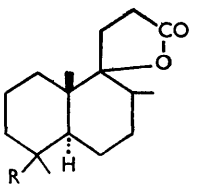
To substantiate the conclusions reached so far we wished to correlate marrubiin with some substance of known structure.[6] Removal of the oxygen at position 8 (of marrubiin; cf. XXI) by the toluenesulphonate method seemed unpromising (see above), and attempted Wolff–Kishner reduction of the keto-acid (XXII) gave the stable six-membered cyclic hydrazide,[1] so that hydrogenolysis [2] of the enol-lactone of (XXII) was re-investigated. The main reaction product (70%) was the acid lactone (XXIII), but, contrary to the findings of Cocker *et al.*,[2] the minor product (20%) was the dilactone (IIa) and not unchanged enol-lactone.

[6] Burn and Rigby, *Chem. and Ind.*, 1955, 386.

Thionyl chloride converted the lactonic acid (XXIII) into its acid chloride and this was reduced by the Rosenmund procedure (without the use of a catalyst poison) to give the aldehyde (XXIIIa). This, by the Wolff–Kishner (Huang-Minlon) procedure, gave

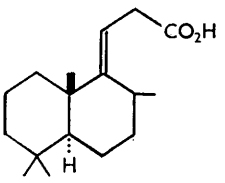


(XXII)



(XXIII): R = CO_2H
(XXIIIa): R = CHO
(XXIIIb): R = CH_3

the expected tetramethyl derivative (XXIIIb) as the minor (25%) product: it was identical with one of the "*iso*ambreinolides" prepared by Collin-Asselineau *et al.*[7] by the action of 70% sulphuric acid on ambreinolide. However, the major product (62%) of the



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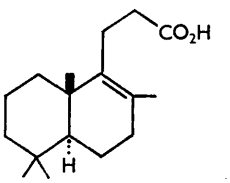

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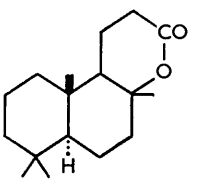

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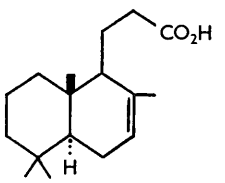
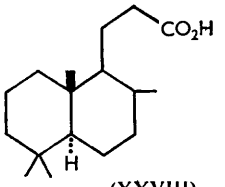


(XXVII)

Wolff–Kishner reaction was an acid $C_{17}H_{28}O_2$, m. p. 122—123·5°, $[\alpha]_D$ +86·3°, which gave a yellow colour with tetranitromethane. Its structure would be expected to be (XXIV) or (XXV). It was not decarboxylated, even at 260°, so that it is not a $\beta\gamma$-unsaturated acid, and its formula will therefore be (XXV). A substance of this structure had been prepared by Ruzicka and Lardon [8] and by Dietrich and Lederer [9] by the action of sulphuric acid on ambreinolide (XXVI), and its structure had been established by Dietrich and Lederer [9] who showed that, in spite of the ease with which it could be hydrogenated, it was not (XXVII). The acid derived from marrubiin was shown to be identical with a sample of the ambrein-degradation product which had been supplied by Professor E. Lederer



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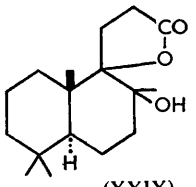

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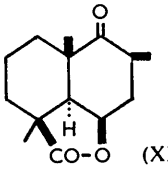

(XXX)

(m. p., mixed m. p., and infrared spectra). This identity was confirmed by comparison of the hydrogenated acids (XXVIII) and by converting the acid derived from marrubiin into the known lactone (XXIX) by osmium tetroxide.

Whether the unsaturated acid should be formulated as (XXV) or (XXVII) is immaterial so far as correlation of the structures of marrubiin and ambrein is concerned, but the formulation (XXV) is perhaps more in keeping with its preparation from marrubiin.

Formation of the unsaturated acid during the Wolff–Kishner reaction was surprising: the lactone (XXIIIb) may be an intermediate, as trial showed that when this is heated

[7] Collin-Asselineau, Lederer, Mercier, and Polonsky, *Bull. Soc. chim. France*, 1950, 720.
[8] Ruzicka and Lardon, *Helv. Chim. Acta*, 1946, **29**, 912.
[9] Dietrich and Lederer, *ibid.*, 1952, **35**, 1148.

with alkali under the conditions of the Wolff–Kishner reduction (but in the absence of hydrazine) the unsaturated acid is produced in poor yield.

The above correlation appears to establish the skeleton (XXI) in marrubiin; the formation of the "*iso*ambreinolide" (XXIIIb), which is known [9] to be a γ-lactone, gives further proof of the position of the tertiary hydroxyl group in marrubiin. The asymmetric centre carrying the bridgehead methyl group in these substances has remained unaffected during the transformations, and so the angular methyl group in marrubiin bears that stereochemical relationship to the ring system which has been found to be general for the resin acids, the triterpenoids, and the steroids. Recovery of some of the dilactone (IIa) in the initial hydrogenation shows that the ring junction is also unaffected, so that marrubiin has a *trans*-ring junction. These are the only established stereochemical features of marrubiin.

Arguments have been advanced [10] purporting to establish the configurations throughout the molecule of marrubiin, but they are unacceptable (although of course any of the conclusions may be true).

Position 6 (numbering as XXI): As the keto-lactone $C_{14}H_{20}O_3$ (I) from anhydromarrubiin or anhydrotetrahydromarrubiin is unchanged by alkali, Cocker, Edward, and Holley [10] infer that the 6-methyl group in marrubiin is equatorial (α). However, if this methyl group were axial in marrubiin, the generated keto-lactone should be (XXX) (on their amended interpretation [10b] of the configuration of the lactone bridge) and it is to be expected that this tri-α-substituted ketone would pass into its $C_{(6)}$-epimer with such ease that failure to isolate it would occasion no surprise.

Position 5: It is argued [10] that because anhydromarrubiin has an exocyclic double bond here the 5-hydroxyl group in marrubiin must be equatorial (β). This tacitly assumes that marrubiin, on dehydration, gives predominantly the product which has the exocyclic double bond. So far as we are aware, there is no published claim of a high yield of anhydromarrubiin or of anhydrotetrahydromarrubiin: we never obtained more than 37%, and the major products are some other uncharacterised (unsaturated) substances. In the absence of evidence that exocyclic dehydration is favoured, any arguments based on that assumption are speculative. Even if it were known that exocyclic dehydration is favoured the argument that the 5-hydroxyl group of marrubiin is β is still unsound, because if the 6-methyl group is β then endocyclic dehydration is unfavoured whether the 5-hydroxyl group is α or β.

Positions 8 and 1: By applying Klyne's lactone rule [11] to the molecular-rotation contribution of the bridging lactone Cocker *et al.*[10] ultimately deduce that the potential 8-hydroxyl group must be β and that, in consequence, the 1-carboxyl group must also be β. There is no reason to believe, however, that this "lactone rule" would be valid even for the structure favoured by Cocker *et al.*—data concerning its application to such structures apparently do not exist.[11] Cocker *et al.* point out [10] that vicinal effects could make the rule of Klyne and Stokes [10c] invalid for this hydroxyl group. They ignore such effects in their stereochemical deductions, even though they believe [2] them to be very strong in a related keto-acid. Furthermore, there is no stereochemical reason for omitting from consideration a "skew" lactone bridge (1α ⟶ 8β or 1β ⟶ 8α), so that the configuration at $C_{(1)}$ does not follow from that at $C_{(8)}$ (or *vice versa*), as has been assumed.[10]

Experimental

Rotations were determined in B.P. chloroform and ultraviolet absorption spectra in ethanol solutions.

Extraction of Marrubium vulgare (White Horehound).—The coarsely chopped, dry, whole plant (12·5 kg.) was extracted continuously with warm acetone for 72 hr. The cold extract (5 l.) was filtered from a crystalline wax *A*, and acetone was removed. A benzene solution of

[10] Cocker, Edward, and Holley, *Chem. and Ind.*, (*a*) 1954, 1561; (*b*) 1955, 772; (*c*) cf. Klyne and Stokes, *J.*, 1954, 1979.
[11] Klyne, *ibid.*, 1954, 1198.

the residue was decanted from some syrup (from which potassium nitrate crystallised); 2% of alcohol was added and the solution was poured through a heated column of silica (1250 g.). The marrubiin was contained in the first 8 l. of eluate; further elution with benzene containing 5% of alcohol gave successively a golden-yellow substance *B*, and a substance *C*; elution with methanol yielded a substance *D*.

Marrubiin. The eluate was concentrated to 1 l. and the marrubiin which crystallised (206 g.) was washed with benzene. The green product was boiled for 5—10 min. with alcohol (250 ml.) and 10N-sodium hydroxide (25 ml.) (in which it did not dissolve); there was negligible loss and the pale green product, m. p. 154—156°, was pure enough for most purposes. Chromatography in benzene on alumina gave pure marrubiin, prisms (from ethyl acetate), m. p. 160°, $[\alpha]_D$ +35·8° (*c* 1·2), infrared max. at 1780 cm.$^{-1}$ (in CS_2).

Other Substances isolated from Horehound.—The wax *A* [12] was chromatographed on alumina; benzene–light petroleum (1 : 1) eluted hentriacontane, pearly plates (from benzene by addition of alcohol), m. p. 68·5—69·5° (Found : C, 85·15; H, 14·8. Calc. for $C_{31}H_{64}$: C, 85·2; H, 14·8%). The benzene filtrate from which the marrubiin was initially crystallised was evaporated and a light petroleum solution of the residue was extracted with 90% aqueous methanol and evaporated, and the residue was boiled with several portions of methanol. The methanol extracted a wax which was then chromatographed on alumina : benzene containing 1—2% of alcohol eluted β-sitosterol, m. p. and mixed m. p. 137°. The *substance B* crystallised from dioxan in golden needles, m. p. 215° (decomp.), $\lambda_{max.}$ 2180, 2870, 3320 Å (log ε 4·52, 4·39, 4·43) in EtOH; 2040, 2900 Å (log ε 4·55, 4·27) in the presence of sodium hydroxide [Found : C, 64·7; H, 4·9; OMe, 19·3. $C_{15}H_{10}O_4(OCH_3)_2$ requires C, 64·55; H, 5·1; OMe, 19·6%]. The *substance D* crystallised in colourless rosettes (from 2-ethoxyethanol), m. p. 280—282° (Found : C, 72·3; H, 10·5. $C_{17}H_{30}O_3$ requires C, 72·3; H, 10·7%).

Dilactone (IIa) *of* β-(5-*Carboxy*-1 : 4-*dihydroxy*-2 : 5 : 9-*trimethyl*-1-*decalyl*)*propionic Acid.*—(*a*) The lactonic hydroxy-acid (II) (5·5 g.) was heated in nitrogen at 230° until gas evolution ceased (only water was evolved and only a few minutes were needed). The colourless residue was crystallised from ethyl acetate–*cyclo*hexane or from cineole to give the dilactone (yield ~100%) as needles, m. p. 163—164°, $[\alpha]_D^{25}$ +29·3° (Found : C, 68·3; H, 8·15. Calc. for $C_{17}H_{24}O_4$: C, 69·8; H, 8·3%). (*b*) Marrubiin (6 g.) in acetic acid (60 ml.) was added to a solution of chromium trioxide (14 g.) in 85% acetic acid (140 ml.). After 2 days the solution was evaporated, hydrochloric acid (60 ml.) was added, and the syrup which was obtained by ether-extraction was chromatographed on silica (50 g.); benzene containing 2% of alcohol eluted the dilactone (1·35 g.), m. p. and mixed m. p. 163—164°. (*c*) A solution of the lactonic hydroxy-acid (II) (0·5 g.) in acetic anhydride (1 ml.) containing anhydrous sodium acetate (0·05 g.) was boiled for ½ hr. The residue obtained on evaporation was chromatographed on silica (20 g.) : benzene containing 2% of alcohol eluted successively the dilactone (0·13 g.; m. p. 163—164°) and the acetate of the hydroxy-acid (II) (0·3 g.; m. p. 262—263°).

Dilactone (XI) *of* α-*Ethoxalyl*-β-(5-*carboxy*-1 : 4-*dihydroxy*-2 : 5 : 9-*trimethyl*-1-*decalyl*)*propionic Acid.*—A solution of the dilactone (IIa) (1 g.) in ethyl oxalate (4 ml.) was heated with sodium ethoxide in ethanol (5%; 1·6 ml.) at 50—60°/100 mm. for 1 hr. and for a further 2 hr. at 20 mm., the alcohol distilling (cf. Floyd and Miller [13]). The excess of ethyl oxalate was removed at a lower pressure and a methanolic solution of the residue was gradually acidified with dilute sulphuric acid, so that the product crystallised. Recrystallisation from alcohol gave the α-*ethoxalyl-dilactone* (XI) as needles, m. p. 184·5—185°, $[\alpha]_D^{22}$ +36·01° (*c* 1·83), $\lambda_{max.}$ 2740 Å (log ε 4·01) displaced to 3230 Å (log ε 4·04) by sodium hydroxide (Found : C, 63·9; H, 7·4. $C_{21}H_{28}O_7$ requires C, 64·3; H, 7·2%). It gave an intense purple colour with ferric chloride. It gave a 2 : 4-*dinitrophenylhydrazone* (prepared in alcohol–phosphoric acid), yellow needles (from chloroform–alcohol), m. p. 242·5—244° (decomp.) (Found : C, 56·1; H, 5·65; N, 10·1. $C_{27}H_{32}O_{10}N_4$ requires C, 56·6; H, 5·6; N, 9·8%).

Halogenation of the α-*Ethoxalyl-dilactone* (XI).—Bromine was rapidly decolorised (carbon tetrachloride; sunlight), but the halogen-containing oil did not crystallise.

The ethoxalyl-dilactone (0·4 g.) was boiled with sulphuryl chloride (20 ml.) for ½ hr., the solution evaporated, and the residue chromatographed on silica (15 g.). Benzene containing 1% of alcohol eluted an oil in which only slight granular crystallisation occurred even on long storage; the oil gave a precipitate with 2 : 4-dinitrophenylhydrazine. When the oil was

[12] Seppi, *Boll. Chim. farm.*, 1947, **86**, 56.
[13] Floyd and Miller, *J. Amer. Chem. Soc.*, 1947, **69**, 2354.

dissolved in methanol, well-formed lath-like crystals of the *methyl hemiketal* of the α-chloro-α-ethoxalyl-dilactone (XII) (0·1 g.) slowly developed, and these readily recrystallised from methanol (see below) having m. p. 132·5—134·5°, $[\alpha]_D$ +5·37° (*c* 1·01), no considerable absorption at $\lambda > 2050$ Å (Found: C, 57·35; H, 6·8; Cl, 7·8%; OAlk, 1·97 mol. $C_{21}H_{27}O_7Cl,CH_3{\cdot}OH$ requires C, 57·6; H, 6·8; Cl, 7·7%). The substance did not react with 2 : 4-dinitrophenylhydrazine, and solutions of it did not crystallise when seeded with the original granular crystals.

On concentration, the mother-liquors from the crystallisation of the hemiketal yielded the *dilactone* (XIII) (0·1 g.) of α-chloro-β-(5-carboxy-1 : 4-dihydroxy-2 : 5 : 9-trimethyl-1-decalyl)-propionic acid as prismatic needles, m. p. 224·5—226°, $[\alpha]_D^{25}$ +40·42° (*c* 1·01) (Found: C, 61·45; H, 6·6; Cl, 11·55. $C_{17}H_{23}O_4Cl$ requires C, 62·5; H, 7·1; Cl, 10·9%). Recrystallisation of the α-chloro-α-ethoxalyl-dilactone hemiketal from methanol always resulted in partial loss of the ethoxalyl residue and formation of the chloro-dilactone: addition of a trace of hydrochloric acid to the methanol suppressed the hydrolysis but conversion into the chloro-dilactone was complete and rapid in methanol containing a trace of sodium hydroxide.

The chloro-dilactone was recovered after boiling in pyridine or collidine (3 hr.); collidine at 250° (4 hr.) or trimethylamine at 250° (2 hr.) yielded an oil and a substance (10—15%) which crystallised from ether as needles (m. p. 250—255°), was halogen-free, and gave a faint colour with tetranitromethane.

Ozonolysis of the α-Ethoxalyl-dilactone (XI).—Ozonolysis of this lactone (0·3 g.) was carried out in chloroform at −60° until the ferric chloride test was negative (½ hr.). The solution was shaken with ice-water and evaporated: a methanol solution of the residue deposited crystals (0·094 g.), m. p. 192—194° (decomp.). The evaporated mother-liquors were chromatographed on acid-washed silica (10 g.): benzene eluted more (0·08 g.) of the same material, and benzene containing 1% of alcohol then eluted a second substance (0·065 g.), m. p. 230—233°, which crystallised when alcohol was added.

The substance of lower m. p. was recrystallised from chloroform–alcohol to give the mixed *carbonic anhydride* (XVIII), needles, m. p. 195—197° (decomp.), $[\alpha]_D^{21}$ +11·07° (*c* 1·16), $\lambda_{max.}$ 2180 Å (log ε 2·41), 1827 and 1766 cm.$^{-1}$ (Found: C, 63·2; H, 6·75. $C_{17}H_{22}O_6$ requires C, 63·3; H, 6·9%). When the substance was heated at 210—220° carbon dioxide (1·4 mol.) was evolved, but no carbon monoxide. It gave no precipitate with 2 : 4-dinitrophenylhydrazine and no colour with ferric chloride. It was unstable in solution: it could not be recovered from an alcoholic solution which had been kept for 2 days at room temperature.

The substance of higher m. p. was recrystallised from alcohol to give the *dilactone* (XIV) of α-oxo-β-(5-carboxy-1 : 4-dihydroxy-2 : 5 : 9-trimethyl-1-decalyl)propionic acid as prisms, m. p. 235·5—236·5°, $[\alpha]_D^{23}$ −31·32° (*c* 1·01), $\lambda_{max.}$ 2340 Å (log ε 4·02) displaced to 2700 Å (log ε 3·93) by sodium hydroxide [Found: C, 66·3; H, 7·4%; equiv. (at 0°), 301. $C_{17}H_{22}O_5$ requires C, 66·65; H, 7·2%; equiv., 306]. It gave a yellow 2 : 4-dinitrophenylhydrazone, and the violet colour given with ferric chloride was liable to be masked by the formation of an orange precipitate. Its *oxime* (prepared in pyridine) formed prisms (from alcohol), m. p. 259·5—261·5° (decomp.), $\lambda_{max.}$ 2280 Å (log ε 4·03), displaced to 2750 Å (log ε 4·01) by sodium hydroxide (Found: C, 63·8; H, 6·9; N, 4·1. $C_{17}H_{23}O_5N$ requires C, 63·5; H, 7·2; N, 4·4%). Diazomethane at 0° converted the oxime into its *methyl ether*, needles (from ether), m. p. 187·5—188·5° (decomp.), $\lambda_{max.}$ 2790 Å (log ε 4·2) (Found: C, 64·2; H, 7·5; N, 4·4. $C_{18}H_{25}O_5N$ requires C, 64·5; H, 7·5; N, 4·2%).

Lactone (XIX) *of 5-Carboxy-1 : 4-dihydroxy-2 : 5 : 9-trimethyl-1-decalylacetoxamic Acid.*—Hydroxylamine hydrochloride (0·046 g.) was added to a solution of the mixed carbonic anhydride (0·344 g.) in pyridine (0·75 ml.). Vigorous evolution of carbon dioxide occurred immediately (Found: 0·82 mol.). The reaction mixture was acidified and extracted with ether; evaporation of the ether gave the *hydroxamic acid* (XIX), needles (0·13 g.) (from alcohol), m. p. 232·5—234·5° (decomp.) (Found: C, 61·6; H, 8·4; N, 4·65. $C_{16}H_{25}O_5N$ requires C, 61·7; H, 8·1; N, 4·5%). It gave a deep red colour with ferric chloride and an insoluble blue-green complex with copper acetate.

Pyrolysis of the Mixed Carbonic Anhydride (XVIII).—The anhydride (1·1 g.) was heated under nitrogen at 210—220° until gas evolution ceased. The residue, which crystallised, was chromatographed on acid-washed silica (35 g.); benzene eluted 8-*hydroxy*-1 : 6 : 10-*trimethyl*-5-*methylenedecalin*-1-*carboxylic lactone* (XV) (0·48 g.), needles (from aqueous methanol), m. p. 117—118·5°, $[\alpha]_D$ +2·04° (*c* 2·0), $\lambda_{max.}$ 2080 Å (log ε 3·07) (Found: C, 76·5; H, 9·6. $C_{15}H_{22}O_2$ requires C, 76·9; H, 9·5%). It gave only a pale colour with tetranitromethane. Subsequent

elution with benzene containing 1% of alcohol yielded *5-carboxy-4-hydroxy-2 : 5 : 9-trimethyldecalylideneacetic acid 5 ⟶ 4-lactone* (XVII) (0·62 g.), needles, m. p. 229—230° (slow evolution of gas begins a few degrees above the m. p.), $[\alpha]_D^{23}$ +42·57° (*c* 1·01), $\lambda_{max.}$ 2210 Å (log ε 4·11) (Found: C, 68·9; H, 7·95. $C_{16}H_{22}O_4$ requires C, 69·0; H, 8·0%). It gave no colour with tetranitromethane. Its *methyl ester* (diazomethane) formed needles (from aqueous methanol), m. p. 169·5—170·5°, $[\alpha]_D^{22}$ +64·9° (*c* 1·64), $\lambda_{max.}$ 2240 Å (log ε 4·26) (Found: C, 69·9; H, 8·3. $C_{17}H_{24}O_4$ requires C, 69·8; H, 8·3%).

Ozonolysis of the Olefinic Lactone $C_{15}H_{22}O_2$ (XV).—After ozonolysis in methylene dichloride at −60°, the solvent was removed and the residue was decomposed with potassium ferrocyanide solution and steam-distilled. The steam-distillate contained formaldehyde (identified as its dimedone compound, m. p. and mixed m. p. 187—188°), and by the colour reaction with phenylhydrazine hydrochloride–potassium ferricyanide). The residue was extracted with chloroform which, on evaporation, yielded 8-hydroxy-1 : 6 : 10-trimethyl-5-oxodecalin-1-carboxylic lactone (I), needles (from methanol), m. p. 195—196·5° undepressed on admixture with material prepared from anhydrotetrahydromarrubiin (Found: C, 71·4; H, 8·5. Calc. for $C_{14}H_{20}O_3$: C, 71·2; H, 8·5%).

Ozonolysis of the Unsaturated Acid-lactone $C_{16}H_{22}O_4$ (XVII).—After ozonolysis in methylene dichloride at −20°, ice-water was added and the mixture was steam-distilled. The keto-lactone (I) crystallised from the residual liquid. It formed needles (from methanol), m. p. and mixed m. p. 194·5—196°.

The Olefinic Lactone $C_{15}H_{22}O_2$ (XV).—The unsaturated acid-lactone (XVII) (0·164 g.) was heated at 240—250°; carbon dioxide was evolved. The residue was chromatographed on acid-washed silica (10 g.), and benzene containing 1% of alcohol eluted this olefinic lactone (0·12 g.), needles, m. p. 111—113° after crystallisation from methanol; the m. p. was raised to 114—116° on admixture with material prepared by pyrolysis of the mixed carbonic anhydride (XVIII).

β-(*5-Carboxy-1-hydroxy-2 : 5 : 9-trimethyl-*Δ^4*-1-octalyl)propionic Lactone* (III).—Powdered toluene-*p*-sulphonyl chloride (0·9 g.) was added with cooling to the lactonic hydroxy-acid (II) (1 g.) in pyridine (0·6 ml.). After the mixture had been kept overnight at room temperature it was acidified with hydrochloric acid. Extraction with chloroform gave an oil from which, on addition of alcohol, the unsaturated *lactone-acid* (III) (0·3 g.) crystallised. The evaporated mother-liquors were chromatographed on silica (10 g.): benzene containing 0·5% of alcohol eluted successively the dilactone (IIa) (0·34 g.), m. p. and mixed m. p. 163—164°, and the unsaturated lactone-acid (III) (0·17 g.), needles (from alcohol), m. p. 249—251° (decomp.), $[\alpha]_D^{25}$ +32·1° (*c* 0·87) (Found: C, 69·8; H, 8·4. $C_{17}H_{24}O_4$ requires C, 69·8; H, 8·3%). It gave only a very pale yellow colour with tetranitromethane and was not reduced when shaken in acetic acid with hydrogen and Adams catalyst. No crystalline products could be obtained after oxidations with ozone, alkaline potassium permanganate (3 atom-equiv. of O), or chromium trioxide–acetic acid (3 atom-equiv. of O).

β-(*1-Hydroxy-2 : 5 : 9-trimethyl-*$\Delta^{5(10)}$*-1-octalyl)propionic Lactone* (V).—The unsaturated lactone-acid (III) (0·15 g.) was heated under nitrogen at 260°; carbon dioxide was evolved. The residue, crystallised from aqueous methanol, gave the unsaturated *lactone* (V) (0·11 g.) as needles, m. p. 121·5—123·5°, $[\alpha]_D^{26}$ +81° (*c* 2·53), $\lambda_{max.}$ 2120 Å (log ε 3·45) (Found: C, 77·65; H, 9·8. $C_{16}H_{24}O_2$ requires C, 77·4; H, 9·7%). It gave an intense yellow colour with tetranitromethane. It was catalytically reduced (Adams catalyst), and the product (m. p. 97·5—99·5°) gave no colour with tetranitromethane.

Oxidation of β-(*1-Hydroxy-2 : 5 : 9-trimethyl-*$\Delta^{5(10)}$*-1-octalyl)propionic Lactone* (V).—(a) *Selenium dioxide.* The lactone (0·1 g.) in dioxan (2 ml.) containing water (2 drops) was boiled for 40 min. with selenium dioxide (0·05 g.). When the product was chromatographed on silica (20 g.), benzene containing 1% of alcohol eluted β-[1 : 2 : 6(or 3) : 7 : 8 : 9-hexahydro-1-hydroxy-2 : 5 : 9-trimethyl-1-naphthyl]propionic lactone (IX), prisms or needles (0·02 g.) (from aqueous methanol). M. p., mixed m. p., and ultraviolet absorption showed its identity with material prepared from the epoxide (VII) (see below).

(b) *Osmium tetroxide.* Aqueous osmium tetroxide (1%; 10 ml.) was added to the unsaturated lactone (0·1 g.) in *tert.*-butyl alcohol (4 ml.). After reaction had taken place, the osmium complex was decomposed: the best method consisted in passing hydrogen sulphide into a solution in 80% aqueous dioxan; alum (1 mg.) in a few drops of water was added, and osmium sulphide removed on the centrifuge. The oily product was chromatographed on

silica (25 g.). After removal of a small amount of a blue substance with benzene containing 1% of alcohol, benzene containing 5% of alcohol eluted the *γ-lactone* (VIII) of β-(1 : 5 : 10-trihydroxy-2 : 5 : 9-trimethyl-1-decalyl)propionic acid, which formed prisms (from acetone), m. p. 189·5—190·5°, $[\alpha]_D^{16}$ −29·5° (*c* 1·2) (Found : C, 67·3; H, 9·3. $C_{16}H_{26}O_4$ requires C, 68·05; H, 9·3%).

(c) *Potassium permanganate.* A 4% solution of potassium permanganate in 50% aqueous acetic acid (22 ml.) was added to one of the unsaturated lactone (1·04 g.) in acetic acid (10 ml.). After ½ hr. the solution was just decolorised with sulphur dioxide and evaporated, and the residue was shaken with water and chloroform. The material extracted by the chloroform was chromatographed on silica (30 g.); benzene containing 1% of alcohol eluted β-(5 : 10-*epoxy*-1-*hydroxy*-2 : 5 : 10-*trimethyl*-1-*decalyl*)*propionic lactone* (VII), needles (0·42 g.) (from ether–light petroleum), m. p. 136—137°, $[\alpha]_D^{26}$ +7·5° (*c* 1·66) (Found : C, 72·6; H, 9·4. $C_{16}H_{24}O_3$ requires C, 72·7; H, 9·15%).

β-[1 : 2 : 6(or 3) : 7 : 8 : 9-*hexahydro*-1-*hydroxy*-2 : 5 : 9-*trimethyl*-1-*naphthyl*]*propionic Lactone* (IX).—A solution of the epoxide (VII) (0·8 g.) in 85% alcohol (15 ml.) containing sulphuric acid (3 drops) was boiled for ¾ hr. The *diene-lactone* (IX) crystallised when the solution cooled, and more was obtained when the solution was neutralised and concentrated (total yield 0·63 g.); from methanol it formed prisms (slow crystallisation) or needles (rapid crystallisation), m. p. 107—108°, $[\alpha]_D^{21}$ −292·2° (*c* 0·98), $\lambda_{max.}$ 2300, 2370, 2440 Å (log ε 4·25, 4·29, 4·09) (Found : C, 77·8; H, 9·0. $C_{16}H_{22}O_2$ requires C, 78·0; H, 9·0%). It gave an orange colour with tetranitromethane. Boiling with selenium dioxide in dioxan gave a low yield of a substance, m. p. 165—167°, which was eluted from silica by benzene containing 0·5% of alcohol. The ultraviolet absorption spectrum remained unchanged when the diene-lactone was boiled for 15 hr. with sulphuric–acetic acid (1 : 4). The diene-lactone was heated with sodium ethoxide (7 mol.) in diethylene glycol for 5 hr. at 200°; ether-extraction of the diluted solution gave an oil the ultraviolet absorption spectrum of which had no triple peak in the 2300—2450 Å region but had a maximum at 3120 Å.

The Diene-diol $C_{16}H_{26}O_2$ (X).—The diene-lactone (IX) was boiled for 1·5 hr. with lithium aluminium hydride (1 mol.) in ether. Working up in the usual way gave 3-(1 : 2 : 6 : 7 : 8 : 9-*hexahydro*-1-*hydroxy*-5 : 9-*dimethyl*-1-*naphthyl*)*propan*-1-*ol* (X), needles (from acetone), m. p. 100—101·5°, $[\alpha]_D^{26}$ −132·6° (*c* 1·7), $\lambda_{max.}$ 2300, 2380, 2460 Å (log ε 4·24, 4·28, 4·08) (Found : C, 76·3; H, 10·6. $C_{16}H_{26}O_2$ requires C, 76·75; H, 10·5%). It gave an orange colour with tetranitromethane, and a p-*nitrobenzoate*, flakes (from light petroleum), m. p. 92—93° (Found : C, 69·0; H, 7·3; N, 3·6. $C_{23}H_{29}O_5N$ requires C, 69·15; H, 7·3; N, 3·5%).

When the diene-diol was boiled with phosphorus trichloride in benzene an oil was formed which had an ultraviolet absorption spectrum similar to that of the diene-diol.

Dilactone of β-(5-*Carboxy*-1 : 4-*dihydroxy*-2 : 5 : 9-*trimethyl*-Δ³-1-*octalyl*)*propionic Acid.*—A solution of the keto-acid (XXII) (5·37 g.) in acetic anhydride (55 ml.) was boiled for 2 hr. under nitrogen, sodium acetate (0·04 g.) was added, and boiling was continued for a further 2 hr. The solution was evaporated to dryness (low pressure) and a chloroform solution of the residue was washed with aqueous sodium carbonate. Removal of the chloroform gave the enol lactone,[2] prisms (3·44 g.) (from ethyl acetate), m. p. 141—142°, $[\alpha]_D^{20}$ −138° (*c* 1·68), infrared max. at 1778, 1757, 1700 cm.$^{-1}$ (in $CHCl_3$) and 847 cm.$^{-1}$ (in Nujol) (Found : C, 70·2; H, 7·75. Calc. for $C_{17}H_{22}O_4$: C, 70·3; H, 7·6%).

Hydrogenolysis of the Enol Lactone $C_{17}H_{22}O_4$.—The enol lactone (0·5 g.) was hydrogenated in acetic acid (platinum oxide, 1 atm., room temp.). The solvent was evaporated and a chloroform solution of the residue was extracted with dilute sodium hydroxide solution. Evaporation of the chloroform yielded the dilactone (IIa) (0·14 g.), m. p. and mixed m. p. 162—163°. Acidification of the alkaline extract gave the γ-lactone (XXIII) (0·39 g.) of β-(5-carboxy-1-hydroxy-2 : 5 : 9-trimethyl-1-decalyl)propionic acid as prisms, m. p. 273·5—275·5° (lit.,[3] 262—264°), $[\alpha]_D^{21}$ −4·24° (*c* 2·79) (Found : C, 68·8; H, 8·9. Calc. for $C_{17}H_{26}O_4$: C, 69·4; H, 8·9%), the *methyl ester* (prepared by diazomethane) of which formed plates, m. p. 134—136°, $[\alpha]_D^{21}$ 0° ± 0·75° (*c* 2·06) (from methanol) (Found : C, 70·2; H, 9·3. $C_{18}H_{28}O_4$ requires C, 70·1; H, 9·15%).

β-(5-*Formyl*-1-*hydroxy*-2 : 5 : 9-*trimethyl*-1-*decalyl*)*propionic Lactone* (XXIIIa).—(*a*) Purified thionyl chloride (0·5 ml.) and pyridine (1 drop) were added to a suspension of the acid (XXIII) (1 g.) in anhydrous ether (8 ml.) at 0°. After 4·5 hr. at room temperature all the solid had dissolved and the acid chloride was crystallising. The solution was evaporated and the crystalline residue was kept at 100° for 1 hr. under reduced pressure.

(*b*) A solution of this acid chloride in xylene (5 ml.) containing 5% palladised barium sulphate (0·2 g.) was boiled and stirred while a brisk stream of hydrogen was passed through it; the theoretical amount of hydrogen chloride was evolved in 2·75 hr. The *aldehyde-lactone* (XXIIIa) crystallised when the xylene was removed in steam and from methanol formed plates (0·83 g.), m. p. 136·5—137·5°, $[\alpha]_D^{20}$ −33·55° (*c* 2·65), $\lambda_{max.}$ 2790 Å (ε 28) (Found: C, 73·0; H, 9·5. $C_{17}H_{26}O_3$ requires C, 73·3; H, 9·4%). It gave a yellow 2 : 4-dinitrophenylhydrazone, and a *semicarbazone*, needles (from methanol), m. p. 232—234° (decomp.) (Found: C, 64·7; H, 9·0; N, 11·1. $C_{18}H_{29}O_3N_3$ requires C, 64·45; H 8·7; N, 12·5%).

Wolff–Kishner Reduction of the Aldehyde-lactone (XXIIIa).—The aldehyde-lactone (4·5 g.), diethylene glycol (50 ml.), alcohol (25 ml.), sodium ethoxide (from 0·45 g. of sodium), and hydrazine (15 ml.) were boiled together for 1½ hr. More sodium ethoxide (from 1·4 g. of sodium in 25 ml. of alcohol) and benzene (8 ml.) were added, the lower-boiling materials were distilled off and the solution was kept at 200° for 5 hr. The solution was diluted with water, acidified, and extracted with chloroform, and the extracted oil was chromatographed on acid-washed silica (80 g.). Elution with benzene gave successively (*a*) β-(2 : 5 : 5 : 9-tetramethyl-Δ^1-1-octalyl)propionic acid (XXV) (2·8 g.), needles (from 95% methanol), m. p. 122—123·5°, $[\alpha]_D^{20}$ +86·3° (*c* 1·16) (Found: C, 77·2; H, 10·75. Calc. for $C_{17}H_{28}O_2$: C, 77·2; H, 10·7%), and (*b*) β-(1-hydroxy-2 : 5 : 5 : 9-tetramethyl-1-decalyl)propionic lactone (XXIIIb) (1·2 g.) which crystallised slowly on the addition of methanol as needles, m. p. 96·5—97·5°, $[\alpha]_D^{26}$ −10·1° (*c* 1·58) (Found: C, 76·9; H, 10·7. Calc. for $C_{17}H_{28}O_2$: C, 77·2; H, 10·7%). The acid gave a strong yellow colour with tetranitromethane, and was identical (mixed m. p. and infrared spectrum) with a sample of an unsaturated acid prepared from ambrein by Professor E. Lederer [9] {m. p. 122·5—124°, $[\alpha]_D^{20}$ +90·9° (*c* 1·69)}. The lactone gave no colour with tetranitromethane and was insoluble in cold dilute aqueous sodium hydroxide. At Professor E. Lederer's suggestion it was compared with a sample of the "*iso*ambreinolide" of m. p. 98° which he had prepared [7] from ambreinolide; the mixed m. p. was 96·5—98°.

Conversion of the Lactone (XXIIIb) ("iso*Ambreinolide*") *into the Unsaturated Acid* (XXV).—A solution of sodium ethoxide (from 0·04 g. of sodium in 1·5 ml. of alcohol) was added to one of the lactone (0·1 g.) in diethylene glycol (1·5 ml.), the alcohol was removed, and the solution was heated at 200° for 5 hr., diluted, acidified, and extracted with chloroform. The extracted oil was chromatographed on silica; benzene eluted an oil (0·04 g.) which, after crystallisation from aqueous methanol, gave the unsaturated acid (XXV), m. p. and mixed m. p. 121—122·5°.

β-(2 : 5 : 5 : 9-*Tetramethyl*-1-*decalyl*)*propionic Acid* (XXVIII).—The unsaturated acid (XXV) (0·2 g.) (prepared from marrubiin) was hydrogenated (Adams catalyst) in acetic acid. Removal of the solvent gave the saturated acid (XXVIII), needles (from methanol) (0·18 g.), m. p. 137·5—138·5°, $[\alpha]_D^{21}$ +37·73° (*c* 2·36) (Found: C, 76·7; H, 11·4. Calc. for $C_{17}H_{30}O_2$: C, 76·6; H, 11·35%). The unsaturated acid (XXV) which had been derived from ambrein was similarly hydrogenated; the product had m. p. 137—138·5°, $[\alpha]_D^{20}$ +40·17° (*c* 2·23). A mixture of the two substances melted at 137·5—138·5°.

γ-*Lactone of* β-(1 : 2-*Dihydroxy*-2 : 5 : 5 : 9-*tetramethyl*-1-*decalyl*)*propionic Acid* (XXIX).—The unsaturated acid (XXV) was hydroxylated with osmium tetroxide in pyridine by the method of Dietrich, Lederer, and Mercier [14] and the product was chromatographed on acid-washed alumina. Benzene containing 0·5% of alcohol eluted the hydroxy-lactone (XXIX), flat needles (from ether–light petroleum), m. p. 142·5—144°, $[\alpha]_D^{20}$ −3·75° (*c* 1·47) (Found: C, 72·8; H, 10·2. Calc. for $C_{17}H_{28}O_3$: C, 72·8; H, 10·1%). Dietrich, Lederer, and Mercier [14] report m. p. 143—144°, $[\alpha]_D$ −4·5° (*c* 1·1), for material prepared from ambrein.

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[14] Dietrich, Lederer, and Mercier, *Helv. Chim. Acta*, 1954, **37**, 705.