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CONSTITUENTS OF *HELENIUM* SPECIES—XVIII.

1-EPIISOTENULIN AND ITS TRANSFORMATIONS[1]

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Abstract—The stereochemistry of mexicanin A has been established. The inversion of the asymmetric center at C_1 of isotenulin is described and the lack of correspondence between the ORD curves of *cis*-fused 4-ketopseudoguaianolides and 14β-17-ketosteroids is discussed. The course of a remarkable transformation of 1-epidihydroisotenulin has been elucidated.

IN AN earlier paper[2] we reported the conversion of helenalin (Ia)[3] to mexicanin A (IIa) by treatment with hydrogen chloride-chloroform. But while the relative and absolute stereochemistry of helenalin has since been settled,[5,6] the configuration of mexicanin A at C_5 remained in doubt. The ORD curve of tetrahydromexicanin A (now known to be IIIa-*vide infra*) exhibited[2] a negative Cotton effect antipodal in sign, though not in magnitude, to the strong positive Cotton effect displayed by tetrahydrohelenalin (IVa) and other *trans*-fused hydrogenated pseudo-guaianolides[7] and by 17-keto-steroids.

Since it had been shown conclusively[5] that tetrahydromexicanin A (IIIa) differed from IVa at C_1, this inversion of the Cotton effect suggested that the conversion of Ia (C_5-methyl β) to IIa involved not only double bond migration, but also epimerization at C_5 by way of an acid-catalyzed retroaldol reaction. Additional support for this conclusion was apparently forthcoming from our unsuccessful efforts[2] to obtain acetylmexicanin A (IIb) by treatment of acetylhelenalin (Ib), with hydrogen chloride-chloroform.

Repetition of this work has shown this conclusion to be incorrect. Exposure of Ib to hydrogen chloride-chloroform and analysis of the crude reaction mixture by TLC and NMR spectroscopy established the presence of some acetylmexicanin A which must therefore have configuration IIb, although the equilibrium Ib $\rightleftharpoons$ IIb lies farther to the left of the equilibrium Ia $\rightleftharpoons$ IIa. Tetrahydromexicanin A is therefore IIIa. Obviously comparison of the ORD curve of IIIa with the rigid ring system of 17-ketosteroids was not warranted.

This conclusion was placed on a secure footing by studying the same reaction in the isotenulin series. Treatment of isotenulin (Vb) with hydrogen chloride-chloroform

[1] Supported in part by grants from the National Science Foundation (GP-1492) and the U.S. Public Health Service (GM-05814). Previous paper, W. Herz and M. V. Lakshmikantham, *Tetrahedron* **21**, 1711 (1965).

[2] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *J. Amer. Chem. Soc.* **85**, 19 (1963).

[3] The gross structure of helenalin was deduced in Ref. 2, the relative and absolute configuration of helenalin and its congeners in Refs. 4–6.

[4] D. Rogers and Mazhar-ul-Haque, *Proc. Chem. Soc.* 92 (1963).

[5] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *Tetrahedron* **19**, 1359 (1963).

[6] M. T. Emerson, C. N. Caughlan and W. Herz, *Tetrahedron Letters* 621 (1964).

[7] C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.* **22**, 1361 (1957).

resulted in isomerization to an analog (VI) of IIb whose physical properties (composite IR band at 1770 cm^{-1}, enhanced UV absorption—ε 260 at 278 mμ) suggested that it was a β,γ-unsaturated ketone. The NMR spectrum (broad triplet at 6·00 ppm, —H_2, pair of doublets at 3·05 and 2·92 ppm —H_2) was similar to that of IIb and the conversion of VI to desacetylneotenulin[8] on treatment with sodium bicarbonate was analogous to the conversion of IIa to neohelenalin.[2] Further treatment of VI with methanol-hydrochloric acid resulted in regeneration of the α,β-unsaturated cyclopentenone chromophore and formation of a substance isomeric but not identical with isotenulin. The UV, IR and NMR spectrum were in accord with gross structure VII and since the only asymmetric center involved in this transformation is C_1, the new substance had to be 1-epiisotenulin (VII)[9,10].

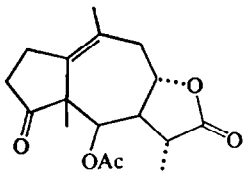
Catalytic reduction of VII in ethanol in the presence of Pd-C yielded 1-epidihydroisotenulin VIIIb which was also obtained in excellent yield by reduction of VI with PtO_2 in acetic acid. The ORD curves of VII and VIIIb exhibited positive and negative Cotton effects respectively, the latter coinciding with that of IIIa, which are exactly the opposite of those shown by isotenulin (V) and dihydroisotenulin (IXb),[7] respectively. This observation confirms the postulated difference in the stereochemistry at C_1 and again indicates that the ORD curves of *cis*-fused pseudoguaianolides of type III and VIII are not comparable to those of 14β-17-ketosteroids.

That the "Cyclopentenone Rule[11]" can be applied to all compounds of type A[5,7] is apparently due to the fact, demonstrable with models, that in A the cyclopentenone ring is forced into a conformation with the "right" chirality whatever the conformation of the seven-membered ring. On the other hand, models show that in compounds of type B, of which VII is the first example, the cyclopentenone ring might be flat for many conformations of the seven-membered ring and that it would be difficult to

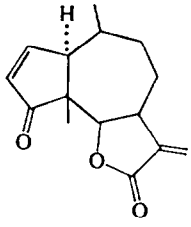
[8] W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman and N. Viswanathan, *J. Amer. Chem. Soc.* **84**, 3857 (1962).

[9] Treatment of helenalin or mexicanin A with MeOH–HCl results in further transformations which will be detailed in a subsequent publication.

[10] This conclusion is confirmed by the ORD curves and the chemical transformations which are discussed subsequently. While it is conceivable that C_{10} might have been inverted as well through the transitory existence of i, this possibility is considered unlikely because we are not aware that γ,δ-unsaturated ketones have previously been implicated as intermediates in acid-catalyzed equilibrations of α,β- and β,γ-unsaturated ketones and because the presence of i could not be demonstrated when crude reaction mixtures were analyzed at various time intervals. Moreover, there is conclusive evidence that the stereochemical integrity at C_{10} of ambrosin (ii) is maintained during a similar series of transformation (to be published).



i



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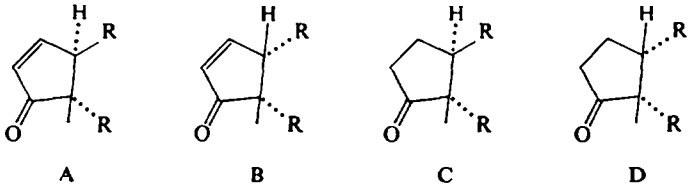
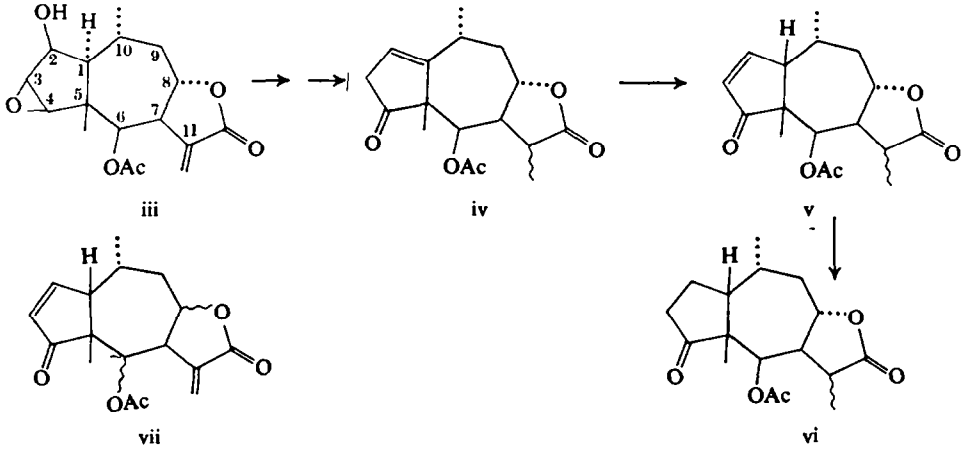
[11] G. Snatzke, *Tetrahedron* **21**, 421 (1965). We are indebted to Dr. Snatzke for correspondence dealing with this matter.

predict the sign of the Cotton effect although the ORD curve of VII resembles that of Δ^{16}-14β-17-ketosteroids. Analogously saturated *trans*-fused pseudoguaianolides (type C) have a nearly fixed conformation of the five-membered ring and therefore exhibit Cotton effects of the same sign as steroidal ketones of comparable structure.[5,7] Contrariwise, in *cis*-fused saturated pseudoguaianolides (type D) there is no fixed conformation similar to that adopted by 14β-17-ketosteroids and each compound must be considered *sui generis*.[12]

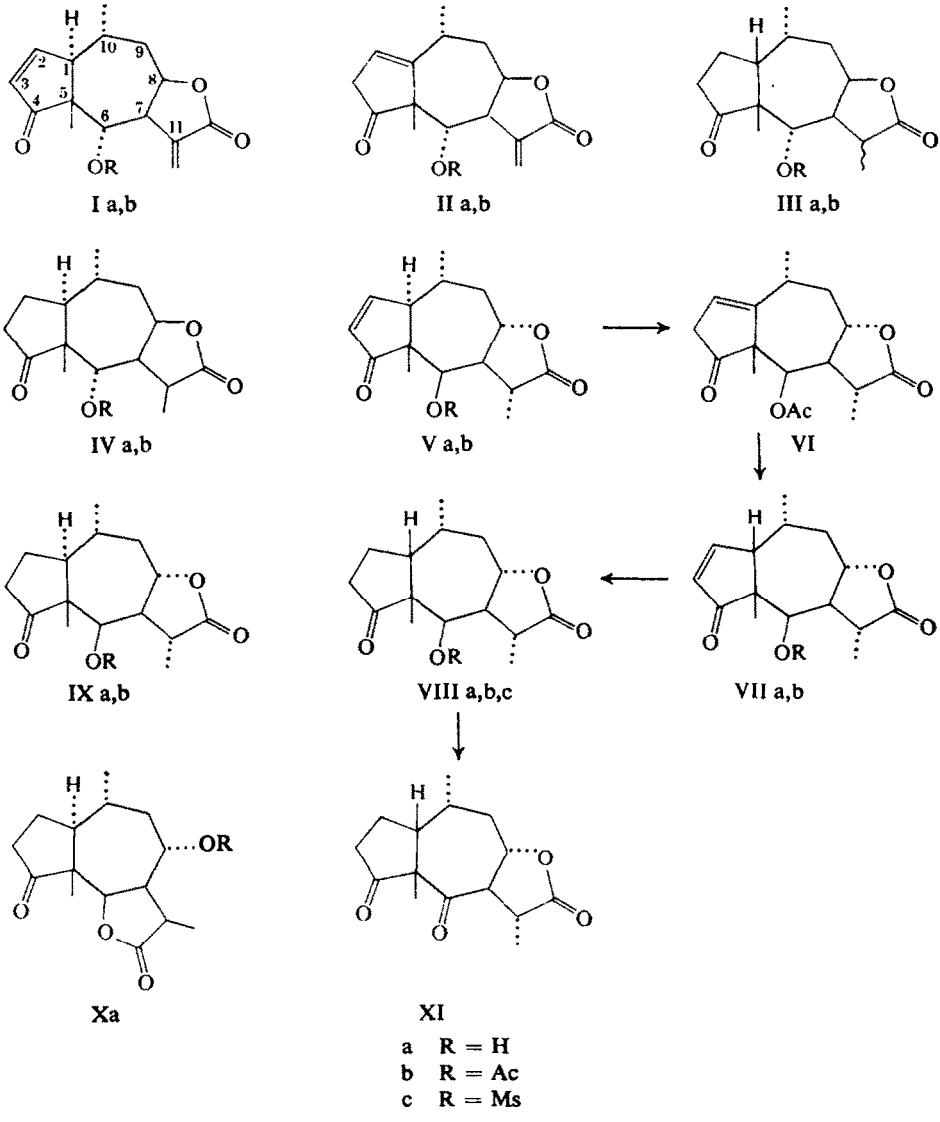


We now wish to consider a remarkable transformation of VIIIb which seems to be intimately bound up with the *cis*-fusion of the two alicyclic rings and indeed furnishes strong additional evidence for the postulated change in the stereochemistry of the ring junction. In an effort to remove the functional groups of VIIIb gradually and to correlate the new epimer of dihydroisotenulin with other pseudoguaianolides whose

[13] It is interesting to apply our observations in the 1-*epi* isotenulin series to two epimers of isotenulin which we have described previously. One of these is a substance (v) which was prepared by acid-catalyzed equilibration of an epimer iv of VII which was in turn obtained from gaillardilin (iii).[13] The change in Cotton effect accompanying the reduction of v to vi parallels[14] that observed during the reduction of VII to VIIIb. Hence we conclude that v and vi are *cis*-fused, with H_1 and C_6-methyl β since the stereochemistry at C_7 and C_{10} is probably that invariably encountered in other compounds isolated from *Helenium and Gaillardia* species. For the same reason we tentatively assign to linifolin A[14] the *cis*-fused structure vii. It exhibits a relatively weak multiple positive Cotton effect curve which on reduction to tetrahydrolinifolin A undergoes inversion to negative sign. Tetrahydrolinifolin A differs from the seven known *trans*-fused C_6, C_8 and C_{11}-epimers of IVb as well as from VIIIb and from vi.



[13] W. Herz, S. Rajappa, M. V. Lakshmikantham and J. Schmid, *Tetrahedron* **22**, 693 (1966).
[14] W. Herz, *J. Org. Chem.* **27**, 4043 (1962).



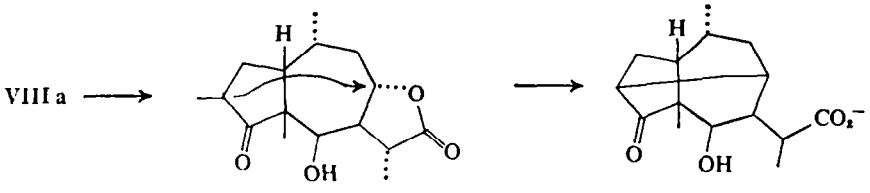
stereochemistry was still in question, it was subjected to the action of base. Treatment with potassium carbonate in boiling methanol did not furnish the expected VIIIa which was later obtained in low yield by acid hydrolysis (dioxan–HCl) but resulted in isolation of three substances X($C_{15}H_{22}O_4$), Y($C_{15}H_{20}O_3$) and Z($C_{15}H_{20}O_3$) in 45–50%, 5% and 2% yield. The remaining material consisted of gums which have so far resisted attempts at purification.

Although the major product X had the formula $C_{15}H_{22}O_4$, it was clearly not a simple deacetylation product of VIIIb. Its solubility in base and IR spectrum (broad band at 3500 and 1705 cm^{-1}) established it as a carboxylic acid which was confirmed by conversion to a methyl ester. A third oxygen atom was that of a cyclopentanone carbonyl as verified by the UV spectra of X and its derivatives, which had a maximum

near 290 mμ, and by the IR absorption near 1745 cm^{-1}. The fourth oxygen atom was part of a hydroxyl group whose presence was betrayed in the IR spectrum of the methyl ester and confirmed by the formation of an acetate.

The facile formation of this acetate suggested that the hydroxyl group was secondary. Inspection of the NMR spectrum revealed a somewhat broadened doublet at 4·08 ppm (J = 8), 3·91 ppm in the NMR spectrum of the methyl ester, which had to be ascribed to hydrogen *alpha* to the hydroxyl group since it moved to 5·00 ppm (J = 10) in the NMR spectrum of the methyl ester acetate. No other low field protons, other than the signal of the acidic hydrogen of X which disappeared on deuterium exchange or esterification, were in evidence, but all NMR spectra exhibited signals characteristic of one tertiary methyl group and two secondary methyls.

The absence of a double bond indicated by the usual chemical tests and the resistance toward hydrogenation coupled with the empirical formula, required that X be tricyclic.[15]



E

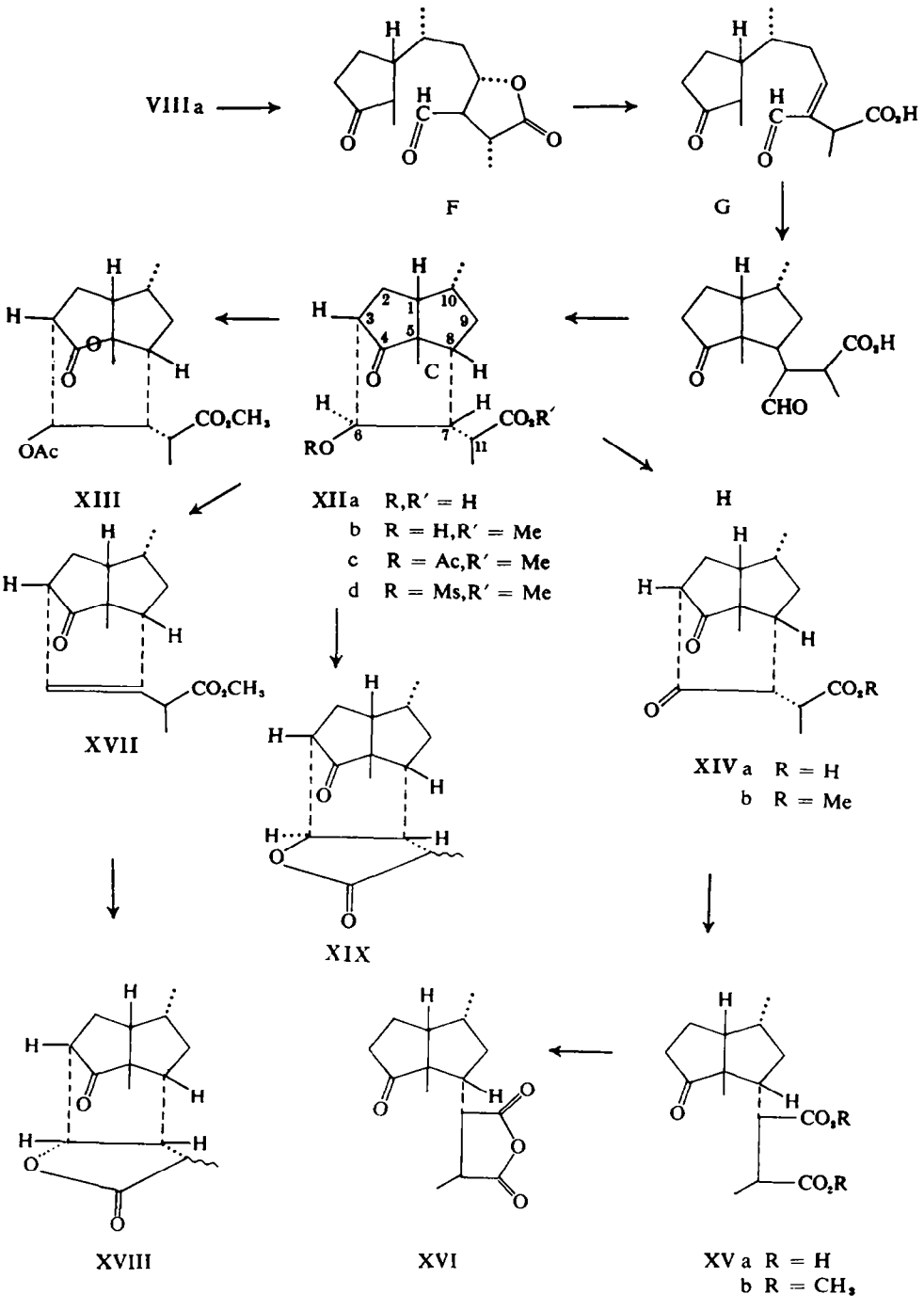
The properties of X suggested initially that it was formed by deacetylation of VIIIb to VIIIa followed by intramolecular nucleophilic displacement of the lactone group by carbanionic attack as illustrated. However although E can be formed with Dreiding models, the models also show that the stereochemistry of the starting material is not at all favorable to the postulated displacement at C_8. Moreover, a number of transformations to be discussed subsequently could not be interpreted in terms of E.

On the other hand the working hypothesis XIIa could be confirmed in subsequent work. Such a substance could have been formed by initial deacetylation to VIIIa followed by retroaldol reaction to F(Scheme II). Now a substance of type F in the pseudoguaianolide series is prone to undergo an internal base-catalyzed elimination reaction to G[8] which in turn is well disposed toward an intramolecular Michael condensation involving C_5 and C_8. The reversibility of the latter favors formation of the thermodynamically more stable *cis*-isomer H where the inviolability of the β-orientated proton at C_1 assures the depicted absolute stereochemistry at C_1 and C_5. The spatial propinquity of the aldehyde group and C_3 then favors intramolecular aldol condensation to XIIa, a substance which is easily constructed with Dreiding models

[15] Before possible pathways leading to X and the minor products Y and Z are considered, it must be borne in mind that the hydrolysis of dihydroisotenulin (IXb) itself proceeds normally. The product is desacetyldihydroisotenulin (IXa) mixed, if the hydrolytic conditions are somewhat more vigorous, with desacetyldihydroalloisotenulin (Xa), a substance in which the lactone ring has become reorientated toward C_6.[8] In the process the center at C_{11} has also undergone epimerization.[5] The behaviour of helenalin[3,5] and balduilin[5] derivatives is qualitatively similar.

The adoption of an entirely different mode of hydrolytic cleavage in the present instance is further evidence for the conclusion that hydrogen chloride treatment of isotenulin results in epimerization at C_1 and the formation of a *cis*-fused pseudoguaianolide ring system where previously unobserved reactions may supervene during hydrolysis.

Scheme II


VIIIa
F
G
XIII
XIIa R,R′ = H
b R = H,R′ = Me
c R = Ac,R′ = Me
d R = Ms,R′ = Me
XIV a R = H
b R = Me
XVII
XIX
XVIII
XVI
XV a R = H
b R = CH3

if H_3 and H_8 are β with respect to ring A and B respectively. Otherwise formation of ring C is impossible. The stereochemistry at the remaining centers C_6, C_7 and C_{11} will be discussed subsequently. The other alternative, an intramolecular Michael addition of C_3 to C_8 followed by aldol ring closure of the aldehyde function leads to E which, as already mentioned, is not acceptable.

That the α'-position of X and its derivatives was partially substituted as required by both XIIa and E was indicated by the negative Zimmerman test and failure to condense with reactive aldehydes. Dihydroisotenulin (IXb) and its 1-epimer VIIIb under analogous conditions give positive Zimmerman tests or well-defined condensation products. Indeed, the only matter of concern at this stage was the multiplicity of the signal attributed to what was previously H_6 which appeared as a somewhat broadened doublet in the NMR spectra of XIIa, b and c. However, the expected decrease in the rigidity of the system produced by peracid oxidation of XIIc to a δ-lactone XIII effected a splitting of each doublet component which lent substance to the supposition that one of the hydrogen atoms adjacent to the hydroxyl function of XIIa was coupled only slightly to H_6.

That the secondary hydroxyl group of X was β to the cyclopentanone carbonyl was shown by the following transformation. Chromic acid oxidation of XIIb furnished a gummy diketone XIVb (infrared bands at 1770 and 1740 cm^{-1}). While this gave no ferric chloride test, presumably because of the high energy requirements of its enolate, its formulation as a β-diketone was put clearly in evidence since treatment with sodium carbonate followed by reesterification resulted in a dimethyl ketoester XVb whose NMR spectrum, like that of XIIa, displayed two methyl doublets and one methyl singlet. This series of transformations, if applied to E, should have given rise to a substance containing three secondary methyl groups.

Additional proof for the lower half of the molecule could be adduced by oxidizing XIIa directly. This resulted in a crystalline diketoacid XIVa (IR bands at 3500, 1755 and 1730 cm^{-1}) which was cleaved with dilute base in the usual manner. The resultant dibasic acid XVa on refluxing with acetic anhydride furnished a crystalline substituted succinic anhydride XVI (IR bands at 1855 and 1780) whose NMR spectrum again exhibited two methyl doublets and one methyl singlet. Moreover, XVb and XVI were cyclopentanones (IR bands at 1740 and 1730 cm^{-1}, respectively) in which the α-ketomethylene group had been regenerated as indicated by positive Zimmerman tests. The alternative formula E would not have allowed for this.

Conversion of XIIb to the crystalline mesylate XIId followed by treatment with lutidine led to a relatively unstable anhydro derivative XVII which exhibited the characteristic intensified $n \rightarrow \pi^*$-transition and charge transfer bands of certain β,γ-unsaturated ketones (λ_{max} 212 and 290 mμ, ε_{max} 2630 and 273).[17] Worthy of note was the appearance of a vinyl proton doublet at 5·89 ppm (J = 6) which again disproved the alternative formula derivable from E. On standing or on heating, XVII was converted to a γ-lactone XVIII (negative Zimmermann test, IR bands at 1770 and 1750 cm^{-1}) which proved to be identical with Y, the second hydrolysis product of VIIIb. The NMR spectrum of XVIII, in addition to the usual methyl singlet and two methyl doublets, exhibited a sharp triplet at 5·11 ppm (J = 8) clearly indicating that H_6 was spin-coupled to two vicinal protons.

While the proposed structure XIIa for the hydroxyacid X is clearly compatible

[17] S. F. Mason, *Quart. Rev.* **15**, 287 (1961); *Ibid.* **17**, 30 (1963).

with the foregoing chemical and physical evidence, the relative and absolute stereochemistry at C_6 and C_7 shown in XIIa (the situation at C_1, C_3, C_5 and C_{10} having been discussed earlier) is readily confirmed by the NMR spectra of X and its derivatives. During Michael addition, the bulky group at C_7 would be expected to adopt the thermodynamically more stable equatorial configuration which is α when ring C is a boat.[18] On the other hand, aldol condensation at C_3 should give rise to both epimeric alcohols at C_6 since neither suffers severe steric crowding, although the α-hydroxyl group is more hindered than the β- and the mixture of epimers would favor the latter.

A Dreiding model of XIIa shows that H_6 forms angles of 100° and 170° with H_3 and H_7 respectively; this is the only one of four possible configurations which is compatible with the observed doublet ($J = 9{\cdot}5$), the coupling between H_6 and H_3 being observed only by the broadening of each doublet component ($w_{1/2}$ 2·5). The relative stereochemistry at H_6 and H_7 shown in XIIa was confirmed particularly well in two derivatives. First, XIIa on treatment with acetic anhydride formed a γ-lactone XIX, isomeric with XVIII, whose Dreiding model showed that H_6 now formed angles of 105 and 180° with H_3 and H_7 respectively. While the increase from 100 to 105° was not sufficient for resolution, the increase from 170° to 180° between H_6 and H_7 was quite significant and was compatible with the observed broadened doublet ($J = 11$). Secondly, in the NMR spectrum of XIII H_6 was resolved into a doublet of doublets ($J = 10, 1$) indicative of the decrease in ring strain and increase in angle between H_3 and H_6 to 110°.

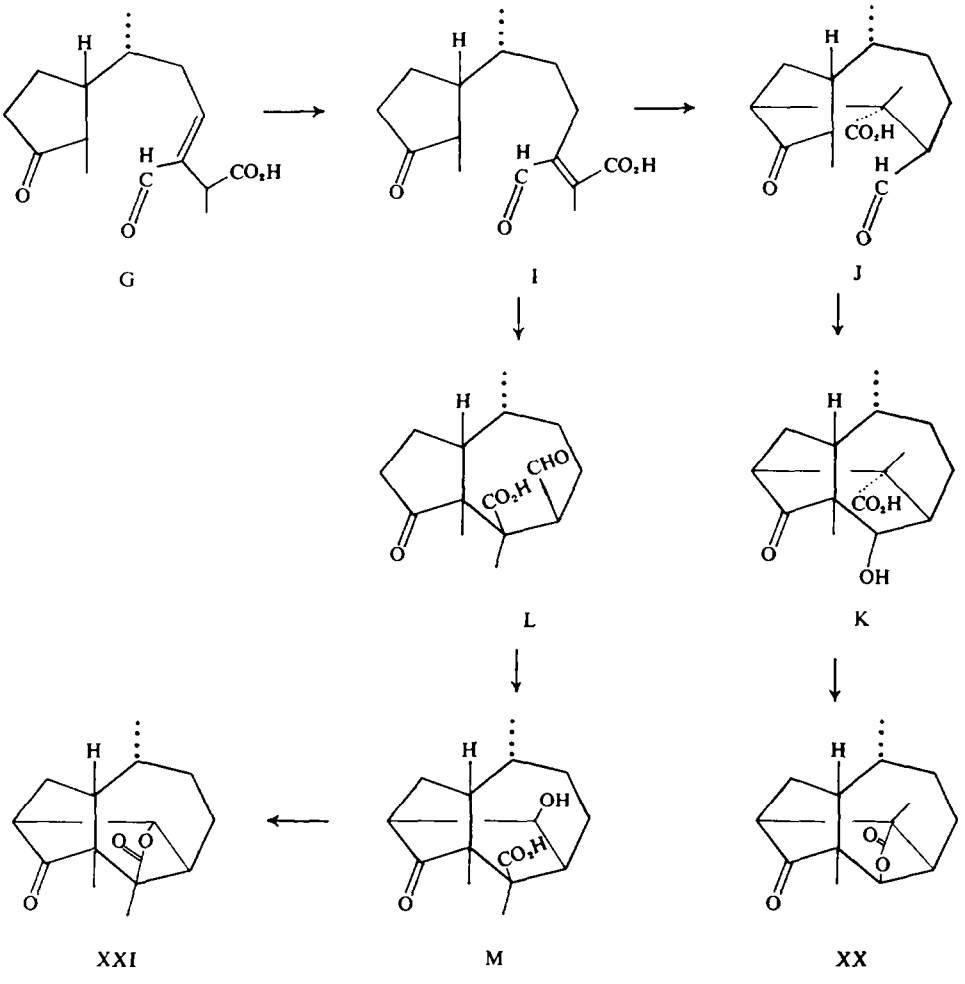
Lactone XVIII represents the other mode of aldol condensation corresponding to the C_6-epimeric alcohol which presumably cyclized spontaneously to the *cis*-lactone. A Dreiding model indicates that H_6 forms angles of 40° and 30° with H_3 and H_7 respectively and is hence consistent with the observed triplet ($J = 8$). The configuration at C_{11} in all compounds remains uncertain.

That lactones XVIII and XIX were indeed epimeric at C_6 was demonstrated in the following way. When XIX was refluxed with aqueous methanolic potassium carbonate under conditions similar to the hydrolysis of VIIIb, the products consisted of XIIa (45%) and XVIII (16%) after acidification. This result is clearly consistent with the known reversible nature of the aldol condensation. On the other hand, hydrolysis of XVIII followed by acidification resulted in XIIa and recovery of XVIII.

There remained the problem of assigning a structure to compound Z, the third substance isolated from the treatment of VIIIb with base. The very low yield precluded chemical investigation, but its physical properties (IR bands at 1770 and 1750, γ_{max} 295 mμ, ε 39) were in accord with the assumption that it was formed from VIIIb by a series of reactions similar to those leading to XIIa. On the basis of the NMR spectrum which exhibited two methyl singlets and one sharp doublet at 4·41 ($J = 2{\cdot}5$) characteristic of hydrogen on carbon carrying a lactone ether oxygen, formula XX can be assigned with some degree of confidence. This may be envisaged (Scheme III) as arising by Michael addition at C_3 of an isomerized unsaturated aldehyde I to give J and, by aldol condensation at C_5, K which then lactonizes to XX. A Dreiding model of the latter showed an angle of 70° between H_6 and H_7 which was consistent with the sharp doublet observed. The alternative structure XXI formed by way of L and M may

[18] Dreiding models indicate that a boat form of ring C is more stable than a chair form, although the following conclusions hold for both forms.

Scheme III



be eliminated on the ground that H_6 makes angles of 30° and 70° with H_3 and H_7 respectively and would thus be expected to appear as a doublet of doublets.

EXPERIMENTAL[19]

Isomerization of acetylhelenalin

A solution of 1·5 g Ib in 75 ml dry $CHCl_3$ was saturated with dry HCl at 0° and then left overnight. Removal of solvent gave a gum which showed on TLC one main spot corresponding to Ib and a minor spot corresponding to IIb. The NMR spectrum of the crude product indicated the presence of IIb in small amount (10–20%). Chromatography resulted in the recovery of about 75% of Ib, but IIb could not be isolated.

[19] M.ps. are uncorrected. Analyses are by Dr. F. Pascher, Bonn, Germany. IR spectra were run in $CHCl_3$ unless otherwise specified, UV spectra in 95% EtOH, rotations in $CHCl_3$. NMR spectra were run on an A-60 spectrometer in $CDCl_3$ with tetramethylsilane serving as internal standard. Signals are reported in ppm. ORD curves were determined by Dr. L. R. Tether on a Rudolph recording spectropolarimeter. TLC plates were developed with $CHCl_3$–ether 1 : 1.

Isomerization of isotenulin to VI

Dry HCl was bubbled through a solution of 5·9 g isotenulin in 100 ml dry $CHCl_3$ for 30 min. The residue obtained after removal of the solvent crystallized from aqueous MeOH as colorless needles of VI, m.p. 126–127°, yield 4·2 g (71%), $[\alpha]_D$ +10° (*c*, 1·2), IR bands at 1750–1770 cm^{-1} (γ-lactone, cyclopentanone, acetate unresolved), UV maximum at 278 mμ (ε 260), NMR signals at 6·00 broad t (1 proton, H_3, J approx. 2·5), 5·71 br (1 proton, H_6), 4·48 td (1 proton, H_8, J_t = 11, J_d = 5) 3·03 t, 2·90 d (1 proton each, C_8-methylene, J = 1·5, 2·5 respectively), 2·18 s (acetate), 1·31 d, 1·17 d (C_{10}- and C_{11}-methyls, J = 7), 1·05 s (C_5-methyl), ORD (*c*, 0·0625) $[\alpha]_{589}$ +19°, $[\alpha]_{333}$ (peak) +192°, $[\alpha]_{306}$ (trough) +64°, $[\alpha]_{250}$ +1165° (last reading). (Found: C, 66·53; H, 7·28; O, 26·00. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24; O, 26·11%.)

Alkaline treatment of VI

A mixture of 0·3 g VI and 0·5 g $NaHCO_3$ in 40 ml 25% aqueous MeOH was refluxed for 3 hr. The solvents were removed under red. press. and the product isolated by extraction with $CHCl_3$. Crystallization of the residue from acetone–ether gave 0·11 g desacetylneotenulin m.p. 241, identified with authentic material by mixed m.p., IR spectra, and TLC.

1-epi-*Isotenulin* (VII)

A solution of 2·4 g VI in 250 ml MeOH containing 50 ml conc. HCl was refluxed for 7½ hr, then concentrated to about 50 ml, diluted with water, saturated with salt and extracted with both $CHCl_3$ and ether. The combined extracts were washed with brine, dried, and evaporated to give a mobile oil which crystallized on addition of ether–hexane (1:1), m.p. 135–145°. Purification was achieved by chromatography on silica gel. Chloroform eluted 1·3 g VII which crystallized from ether–hexane as colorless prisms, m.p. 170°, $[\alpha]_D$ (EtOH) +17° (*c*, 1·65), IR bands at 1775 (γ-lactone), 1750 (acetate), 1705, 1600 cm^{-1} (cyclopentenone), UV maxima at 225 and 320 mμ (ε, 9500 and 82), NMR signals at 7·83 dd (1 proton, H_2, $J_{2,3}$ = 6, $J_{2,1}$ = 2), 6·25 dd (1 proton, H_3, $J_{3,2}$ = 6, $J_{3,1}$ = 2), 5·36 br (1 proton, H_6), 4·18 td (1 proton, H_8, J_t = 10, J_d = 4), 2·18 s (acetate), 1·30 d (C_{11}-methyl, J = 7), 1·10 d (C_{10}-methyl, J = 7), 1·08 s (C_5-methyl), ORD (*c*, 0·93), $[\alpha]_{600}$ +172°, $[\alpha]_{589}$ +183°, $[\alpha]_{365}$ +477°, $[\alpha]_{365}$ +332°, $[\alpha]_{357}$ +390°, $[\alpha]_{350}$ +378°, $[\alpha]_{343}$ +520°, $[\alpha]_{300}$ +2680°, $[\alpha]_{280}$ +3520° (last reading). (Found: C, 66·57; H, 6·92; O, 26·31. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24; O, 26·11%.)

1-epi-*Dihydroisotenulin* (VIIIb)

(a) *From* VI. A solution of 2·65 g VI in 50 ml AcOH containing 1 g PtO_2 was shaken under H_2 at 20 lb. press. for 5 hr. The catalyst was removed by filtration through celite and the filtrate and washings evaporated under red. press. to give a yellow oil which crystallized on moistening with hexane. Crystallization from acetone–hexane afforded colorless plates of VIIIb, m.p. 184–185°, yield 2·08 g. (79%), $[\alpha]_D$ −13° (*c*, 0·0075), IR bands (Nujol) at 1775 (γ-lactone), 1745 (acetate, cyclopentanone), NMR signals at 5·52 br (1 proton, H_6), 4·31 td (1 proton, H_8, J_t = 10·5, J_d = 5·5), 2·15 s (acetate), 1·16 d (6 protons, overlapping C_{10}- and C_{11}-methyls, J = 7), 1·05 s (C_5-methyl), ORD (*c*, 0·0435) $[\alpha]_{600}$ −, $[\alpha]_{589}$ −, $[\alpha]_{317}$ −1750°, $[\alpha]_{273}$ +2275°, $[\alpha]_{250}$ +1760° (last reading). The substance gave an immediate positive Zimmermann test. (Found: C, 65·88; H, 7·86. Calc. for $C_{17}H_{24}O_5$: C, 66·21; H, 7·85%.)

(b) *From* VII. Hydrogenation of 0·34 g VII in AcOEt with 0·05 g Pd–$CaCO_3$ as catalyst yielded 0·2 g VIIIb after crystallization. Identity with the product described in (a) was confirmed by mixed m.p., TLC and comparison of IR spectra.

Basic hydrolysis of VIIIb

A solution of 5·0 g K_2CO_3 in the minimum of water was added to a solution of 2·5 g VIIIb in 125 ml MeOH and the mixture refluxed for 16 hr, then concentrated and diluted with water. The solution was extracted with $CHCl_3$ to remove a trace of unidentified neutral material. The aqueous layer was acidified with 5N HCl, saturated with salt, and extracted thoroughly with ether. The combined extracts were washed once with water, extracted thrice with NaOH, washed with water, dried, and evaporated to furnish colorless needles of XX which were collected with the aid of ether, m.p. 214–215°, yield 0·05 g (2%), $[\alpha]_D$ (EtOH) −45° (*c*, 0·012), IR bands (Nujol) at 3500 (water of crystallization), 1765–1750 cm^{-1} (broad, cyclopentanone and γ-lactone), IR bands ($CHCl_3$) at

1770 cm^{-1} (cyclopentanone and γ-lactone), UV maximum at 295 mμ (ε 39), NMR signals at 4·41 d (1 proton, H_6, $J_{6,7}$ = 2·5), 1·29 s, 1·23 s (C_{11}- and C_5-methyls), 0·98 d (C_{10}-methyl, J = 6·5), ORD (*c*, 0·052) $[\alpha]_{600}$ −40°, $[\alpha]_{589}$ −54°, $[\alpha]_{313}$ −385°, $[\alpha]_{309}$ −576°, $[\alpha]_{285}$ −207, $[\alpha]_{250}$ −940° (last reading). The Zimmermann test was negative. (Found: C, 72·16; H, 8·19; O, 19·27. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%.)

The alkaline extracts were acidified with 5N HCl, saturated with salt, and extracted thoroughly with ether. The washed and dried extracts were evaporated to give a yellow oil which crystallized from AcOEt–hexane to yield 0·55 g of XIIa, m.p. 165–167°. The analytical sample had m.p. 168°, $[\alpha]_D$ (EtOH) +20° (*c*, 0·0055), IR bands (Nujol) at 3500 (hydroxyl), 2800–2600 (acid hydroxyl), 1745 (cyclopentanone), 1715 cm^{-1} (carboxylic acid), NMR signals at 7·15 s broad (2 protons, O*H* and COO*H* removed on treatment of sample with D_2O), 3·98 d (1 proton, H_6, J = 9·5), 1·13 d, 1·04 d (C_{10}- and C_{11}- methyls, J approx. = 6·5 each), 1·05 s (C_5-methyl). The Zimmermann test was negative. (Found: C, 68·02; H, 8·71; O, 23·71. Calc. for $C_{15}H_{22}O_4$: C, 67·64; H, 8·33; O, 24·03%.)

The mother liquors obtained after removal of XIIa were concentrated, methylated with diazomethane, and chromatographed on silica gel (solvent and eluent $CHCl_3$). This gave XVIII (*vide infra*) and XIIb. Alternatively, the crude hydrolysate was methylated and chromatographed. A typical chromatogram of 3·5 g of crude ester fraction furnished, in the first 60 ml of eluate 0·95 g of gum (one spot on TLC) which on recrystallization from acetone–pet. ether yielded 0·1 g XVIII m.p. 145°, $[\alpha]_D^{24}$ (EtOH) −32° (*c*, 0·008), IR bands 1775 (γ-lactone) and 1750 (cyclopentanone), NMR signals at 5·11 t (1 proton, H_6, J = 8) 1·32 d (C_{11}-methyl, J = 8), 1·1 d (C_{10}-methyl, J = 8) and 1·05 (C_5-methyl), in pyridine 5·15 t (8), 1·2 d (6), 1·06 s, 0·89 d (6·5), ORD (*c*, 0·015) $[\alpha]_{600}$ −53°, $[\alpha]_{589}$ −53°, $[\alpha]_{315}$ −840°, $[\alpha]_{276}$ +973°, $[\alpha]_{265}$ +775° (last reading). (Found: C, 72·35; H, 7·69; O, 19·31. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%.)

Fractions 2 and 3 (25 ml each) yielded 0·3 g gum which on TLC gave the spot characteristic of XVIII but could not be crystallized. Fraction 4·5 (0·5 g gum) gave a small amount of XX. The subsequent fractions were combined (total wt. 1·01 g semisolid) which on crystallization from acetone–pet. ether furnished 0·6 g pure XIIb, m.p. 120°, identical with material prepared by methylation of XIIa (*vide infra*). In runs where the acidific fraction was directly esterified the yield of XIIb approximated 50%.

Acid hydrolysis of VIIIb

A solution of 3 g VIIIb in 75 ml unpurified dioxan and 10 ml conc. HCl was refluxed overnight. Solvents was removed, the residue diluted with water and extracted with $CHCl_3$. The extract was washed with $NaHCO_3$aq, water, dried, concentrated and the residue recrystallized from ether–pet. ether. This furnished 0·42 g starting material, m.p. 175°. The mother liquor furnished 0·955 g solid material (two spots on TLC) m.p. 90°, which was taken up in $CHCl_3$ and chromatographed over acid-washed alumina. The first fraction (50 ml $CHCl_3$) eluted 0·18 g VIIIb, subsequent fractions eluted 0·635 g crude VIIIa, m.p. 125°, which was recrystallized from acetone–pet. ether and then melted at 140°, IR bands at 3650 and 3500 (non-bonded and bonded —OH), 1775 (γ-lactone) and 1735 (cyclopentanone), NMR signals at 4·65 td (1 proton, H_8, J_t = 10·5, J_d = 5·5), 4·04 br (1 proton, H_6), 1·18 s (C_5-methyl) and 1·12 d (C_{10}- and C_{11}-methyl superimposed, J = 7·5). The Zimmermann test was positive. (Found: C, 67·54; H, 8·21; O, 24·26. Calc. for $C_{15}H_{22}O_4$: C, 67·64; H, 8·33; O, 24·03%.)

Reacetylation of VIIIa furnished VIIIb in 90% yield. The mesylate VIIIc prepared in the usual manner, was recrystallized from MeOH and melted at 175°. (Found: C, 55·31; H, 6·64; O, 28·32. Calc. for $C_{16}H_{24}O_6S$: C, 55·80; H, 7·03; O, 27·88%.)

Dehydrodesacetyldihydro-1-epiisotenulin (XI)

To a solution of 0·15 g VIIIa in 3 ml AcOH was added a solution of 0·05 g CrO_3 in 1 ml water. After 1 hr, solvent was removed at red. press., the residue diluted with water, extracted with $CHCl_3$ and the washed and dried extracts concentrated. The residue, wt. 0·11 g, was recrystallized from acetone–pet. ether, m.p. 130° (with previous sintering near 120°) homogeneous on TLC, IR bands at 1775 (γ-lactone), 1745 (cyclopentanone) and 1710 cm^{-1} (rel. weak, cycloheptanone). (Found: C, 67·89; H, 7·52; O, 24·56. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

Derivatives of hydroxyacid XIIa

(a) Methylation of XIIa with diazomethane gave the corresponding XIIb which crystallized from acetone–hexane, m.p. 120°, $[\alpha]_D$ (EtOH) +40 (*c*, 0·6), IR bands at 3600 (hydroxyl), 1745 (cyclopentanone), 1730 cm^{-1} (shoulder) (ester), NMR signals at 3·91 d (1 proton, H_6, J = 9·5), 3·72 s (methoxyl), 1·17 d (C_{11}-methyl, J = 6·5), 1·08 s (C_5-methyl), 1·06 d (C_{10}-methyl, J = 6·5), ORD (*c*, 0·052) $[\alpha]_{600}$ +31°, $[\alpha]_{589}$ +31°, $[\alpha]_{310}$ −1655°, $[\alpha]_{271}$ +2572°, $[\alpha]_{250}$ +2111° (last reading). The Zimmermann test was negative. (Found: C, 68·19; H, 8·18; O, 23·42. Calc. for $C_{16}H_{24}O_4$: C, 68·54; H, 8·63; O, 22·83%.)

(b) Acetylation of the above methyl ester with acetic anhydride–pyridine at 25° for 16 hr gave XIIc which crystallized from acetone–hexane, m.p. 125°, IR bands at 1730–1745 cm^{-1} (cyclopentanone, acetate, ester), no hydroxyl absorption, NMR signals at 5·00 d (1 proton, H_6, J = 9·5), 3·70 s methoxyl), 2·07 s (acetate), 1·17 d (C_{11}-methyl, J = 6·5), 1·09 s (C_5-methyl), 1·08 d (C_{10}-methyl, J = 6·5). (Found: C, 67·67; H, 7·73; O, 24·78. Calc. for $C_{18}H_{26}O_5$: C, 67·06; H, 8·13; O, 24·81%.)

(c) *Lactone* XIX. A solution of 0·25 g XIIa in 5 ml acetic anhydride was refluxed for 1 hr. The cooled solution was treated with aqueous MeOH to decompose the reagent and the mixture was saturated with salt and extracted thoroughly with ether. The combined extracts were washed thrice with aqueous N/2 NaOH, once with water, dried, and evaporated. The residue crystallized from acetone–hexane as colorless prisms XIX, m.p. 135–136°, yield 0·18 g, IR bands (Nujol) at 1780 (γ-lactone), 1745 cm^{-1} (cyclopentanone), no hydroxyl absorption, NMR signals at 4·02 d (1 proton, H_6,$J_{6,7}$ = 11), 1·18 d (C_{11}-methyl, J = 6), 1·12 s (C_5-methyl), 1·09 d (C_{10}-methyl, J = 6·5). The substance did not give a Zimmermann test. (Found: C, 72·75; H, 7·76; O, 19·53. Calc. for $C_{18}H_{30}O_3$: C, 72·55; H, 8·12; O, 19·33%.)

Lactone XIII. A solution of 0·372 g XIIc and 0·25 g *m*-chloroperbenzoic acid in 10 ml $CHCl_3$ was refluxed for 16 hr. The cooled mixture was extracted with $NaHCO_3$aq, washed with water, dried, and evaporated. The residue crystallized from acetone–hexane as colorless prisms, m.p. 182°, NMR signals at 4·87 dd (1 proton, H_6, $J_{6,7}$ = 10, $J_{6,2}$ = 1), 3·75 s (methoxyl), 2·08 s (acetate), 1·57 s (C_5-methyl), 1·20 d (C_{11}-methyl J = 6·5), 1·09 d (C_{10}-methyl, J = 6·5). The substance gave a negative Zimmermann test; the IR spectrum had a very strong band at 1748 cm^{-1} (composite of δ-lactone and two ester functions). (Found: C, 63·40; H, 7·83; O, 28·45. Calc. for $C_{18}H_{26}O_6$: C, 63·88; H, 7·74; O, 28·37%.)

Cleavage of ring C

(a). A solution of 0·2 g XIIb in 5 ml AcOH was treated with a solution of 0·2 g CrO_3 in 1 ml water at 25° for 2 hr. The crude product, isolated by dilution and $CHCl_3$ extraction, was triturated with hot hexane to give 0·15 g XIVb as an oil, homogeneous on TLC, IR bands at 1750 (cyclopentanone), 1730 cm^{-1} (cyclohexanone, ester), no hydroxyl absorption, UV maximum at 288 mμ (ε 110), NMR signals at 3·76 s (methoxyl), 1·27 s (C_5-methyl), 1·16 d (C_{11}-methyl, J = 7), 1·01 d (C_{10}-methyl, J = 6·5). The diketone showed a slow positive Zimmermann test due to cleavage under the basic conditions but a negative ferric chloride test.

The foregoing compound XIVb was heated with 3 ml 2N Na_2CO_3 for 3 hr. The cooled solution was washed with ether, acidified, and the product isolated with $CHCl_3$ as a gum which resisted crystallization. Treatment with diazomethane gave XVb as an oil, IR band at 1735 cm^{-1} (cyclopentanone and esters), NMR signals at 3·70 s, 3·62 s (methoxyls), complex superposition of three methyl signals in 1·22 d (7, C_{11}-methyl), 1·22 (C_5-methyl) and 1·06 d ppm (6, C_{10}-methyl). The substance gave an immediate positive Zimmermann test.

(b). A solution of 0·3 g XIIa in 5 ml AcOH was oxidized with 0·3 g CrO_3 in 1 ml water at 25° for 2 hr. The product was isolated by dilution with water and extraction with AcOEt to give 0·3 g XIVa which crystallized from acetone–hexane, m.p. 170°, IR bands at 1750 (cyclopentanone), 1720 cm^{-1} (cyclohexanone, carboxylic acid), NMR signals at 1·23 s (C_5-methyl), 1·16 d (C_{11}-methyl, J = 6·5), 0·97 d (C_{10}-methyl, J = 6). (Found: C, 68·35; H, 7·47; O, 24·28. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

The foregoing XIVa (0·25 g) was cleaved by base treatment as in (a) above to give 0·24 g gum (XV) which was refluxed with 0·8 ml acetic anhydride for 2 hr. The reagent was removed under red. press. and the residue was crystallized from acetone–hexane to give XVI which was purified

further by sublimation, m.p. 120° IR bands at 1855 (weak), 1780 (strong) (5-membered acid anhydride), 1735 cm^{-1} (cyclopentanone), NMR signals at 3·43 dd (1 proton, H_7, J = 9, 2·5), 1·40 d (C_{11}-methyl, J = 7), 1·16 s (C_5-methyl), 1·05 d (C_{10}-methyl, J = 6). The substance gave an immediate positive Zimmermann test. (Found: C, 63·56; H, 7·83; O, 28·89. Calc. for $C_{18}H_{20}O_4 \cdot H_2O$: C, 63·81; H, 7·85; O, 28·34%.)

Olefinic ester XVII

A solution of 0·22 g XIIb in 2 ml dry pyridine was allowed to stand with 1 ml methanesulfonyl chloride at 0° for 16 hr. The precipitate obtained by pouring the mixture into ice water was collected by filtration and crystallized from acetone–hexane to give XIId, m.p. 110°, yield 0·20 g, NMR signals at 4·83 d (1 proton, H_6, J = 9·5), 3·69 s (methoxyl), 3·06 s (mesylate methyl), 1·18 d (C_{11}-methyl, J = 7), 1.07 s (C_5-methyl), 1·05 d (C_{10}-methyl, J = 6·5). (Found: C, 56·79; H, 6·59; O, 27·24; S, 8·82. Calc. for $C_{17}H_{26}O_6S$: C, 56·97; H, 7·31; O, 26·79; S, 8·93%.)

A solution of 0·183 g of the above mesylate in 2 ml lutidine was refluxed for 8 hr. The solvent was removed under red. press., the residue treated with ice and HCl, and then extracted well with hexane and ether. The oil (0·14 g) obtained on evaporation of the washed and dried extracts showed one major and one minor spot on TLC, the latter corresponding to that of XVIII. The hexane-soluble portion of the oil (major portion) was homogeneous on TLC and yielded olefin XVII which slowly lactonized to XVIII on keeping. Because of this instability, no elemental analysis was attempted but the freshly prepared oil showed UV maxima at 212 and 290 mμ (ε, 2630, 273) (β,γ-unsaturated ketone), IR band at 1745 cm^{-1} (cyclopentanone, ester), no γ-lactone absorption, NMR signals at 5·88 d (1 proton, H_6, $J_{6,8}$ = 8), 3·76 s (methoxyl), 1·21 d (C_{11}-methyl, J = 7), 1·08 s (C_5-methyl), 1·07 d (C_{10}-methyl, J = 6·5).

Hydrolysis of lactone XIX

A solution of 0·1 g K_2CO_3 in 5 ml water was added to 0·09 g lactone XIX in 50 ml MeOH and the mixture refluxed for 4 hr. The solution was concentrated under red. press. diluted with water, acidified, saturated with salt, and extracted with ether. The combined extracts were washed with water, extracted with 1N NaOH, washed with water, dried, and evaporated to give 0·015 g lactone XVIII (16%), m.p. 140–144°, IR identical with that of authentic material described above.

The alkaline extracts were acidified, saturated with salt, and the product isolated by ether extraction. The residue crystallized from AcOEt–hexane to give XIIa (0·04 g 45%) identical with authentic material.

Hydrolysis of lactone XVIII in a similar manner gave XIIa and, in the neutral fraction, recovered XVIII, but no lactone XIX.