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CONSTITUENTS OF *HELENIUM* SPECIES—XVI

THE STRUCTURES OF FLEXUOSIN A AND FLEXUOSIN B[1,2]

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Abstract—Two sesquiterpene lactone constituents of *Helenium flexuosum* Raf., flexuosin A and flexuosin B, have formulae $C_{17}H_{24}O_6$ and $C_{20}H_{28}O_6.H_2O$, respectively. Structures I and XX have been assigned to them.

THE isolation of two new sesquiterpene lactones, flexuosin A and flexuosin B, from *Helenium flexuosum* Raf. was described earlier[3] at a time when the carbon skeleton of constituents of *Helenium* species was formulated incorrectly. We now report experiments which show that flexuosin A and flexuosin B are represented by I and XX, respectively.

Flexuosin A, m.p. 220–221·5°, $[\alpha]_D^{26}$ +12·4° ($CHCl_3$, *c* 4·93) has the empirical formula $C_{17}H_{24}O_6$ which suggests that it is an acetate of an alcohol $C_{15}H_{22}O_5$. This is confirmed by the IR spectrum (KBr pellet, bands at 1720 and 1265 cm^{-1}), by hydrolysis (liberation of acetic acid) and by the NMR spectrum (sharp signal at 2·02 ppm).

Two other oxygen atoms are presumably part of a γ-lactone function conjugated with an exocyclic methylene group (IR bands at 1750 and 1690—KBr pellet—, 1755 and 1650—$CHCl_3$, high intensity absorption at 210 mμ—ε 8750 which disappears on reduction). This is supported by the NMR spectrum (doublets—J = 3—at 6·19 and 5·41 ppm which disappear on reduction) and ozonolysis (liberation of formaldehyde which did not occur in the reduced compounds). The non-volatile noncrystalline fraction from the ozonolysis is obviously an enolic α-ketobutyrolactone,[4] which supports the presence of partial structure A, (the necessity for one hydrogen atom at the γ-position will be apparent from the subsequent discussion) as did the attempted catalytic hydrogenation under acid conditions. This effects isomerization of A to B in the manner previously observed for parthenin[4a] and results in the formation of isoflexuosin A (II). Evidence for this is the UV (λ_{max} 223 mμ, ε 14800), IR (strong double bond at 1660 cm^{-1}) and NMR spectrum of II. The latter is particularly significant in that it displays, in addition to a singlet and doublet methyl signal already present in flexuosin A a vinylic methyl resonance at 1·83 ppm which replaces the

[1] Supported in part by grants from the National Science Foundation (G-14396) and the United States Public Health Service (RG-5814).

[2] Papers XIV and XV, J. Romo, A. Romo de Vivar and W. Herz, *Tetrahedron* **19**, 2317 (1963); W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *Ibid*, **19**, 1359 (1963).

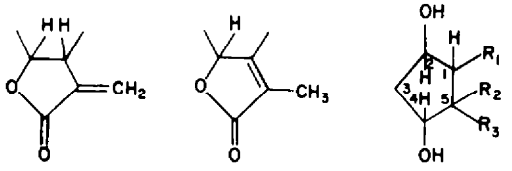
[3] W. Herz, P. Jayaraman and H. Watanabe, *J. Amer. Chem. Soc.* **82**, 2276 (1960).

[4] For analogous results during the ozonolysis of parthenin and pulchellin; [a] W. Herz, H. Watanabe, M. Miyazaki and Y. Kishida, *J. Amer. Chem. Soc.* **84**, 2601 (1962); [b] W. Herz, K. Ueda and S. Inayama, *Tetrahedron* **19**, 483 (1963).

two vinyl doublets of I. Simultaneously, the now allylic signals of H_6 and H_8 shift to lower field (*vide infra*).

The remaining two oxygen atoms of flexuosin A are contributed by two hydroxyl groups (IR bands at 3585 and 3395 cm^{-1}—KBr pellet; conversion to a diacetate) Since only one double bond appears to be present, flexuosin A is assumed to be bicyclic.

That the acetate, the two hydroxyl groups and the lactone ether oxygen are all secondary is suggested by the presence in the NMR spectrum of four low-field signals at 5·98 d (J = 4, acetate),[5] 4·60 td (J = 10·5, 3, lactone), 4·16 c and 3·81 br (5) ppm. In accordance with these assignments, the first two signals stay invariant on conversion of I to its diacetate (III),[6] but the last two signals move downfield to 5·02 td (3, 7·5) and 4·77 d (5) ppm, the multiplicity indicating spincoupling to at least three protons in the former and at least one proton in the latter.



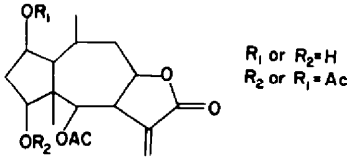
A B C

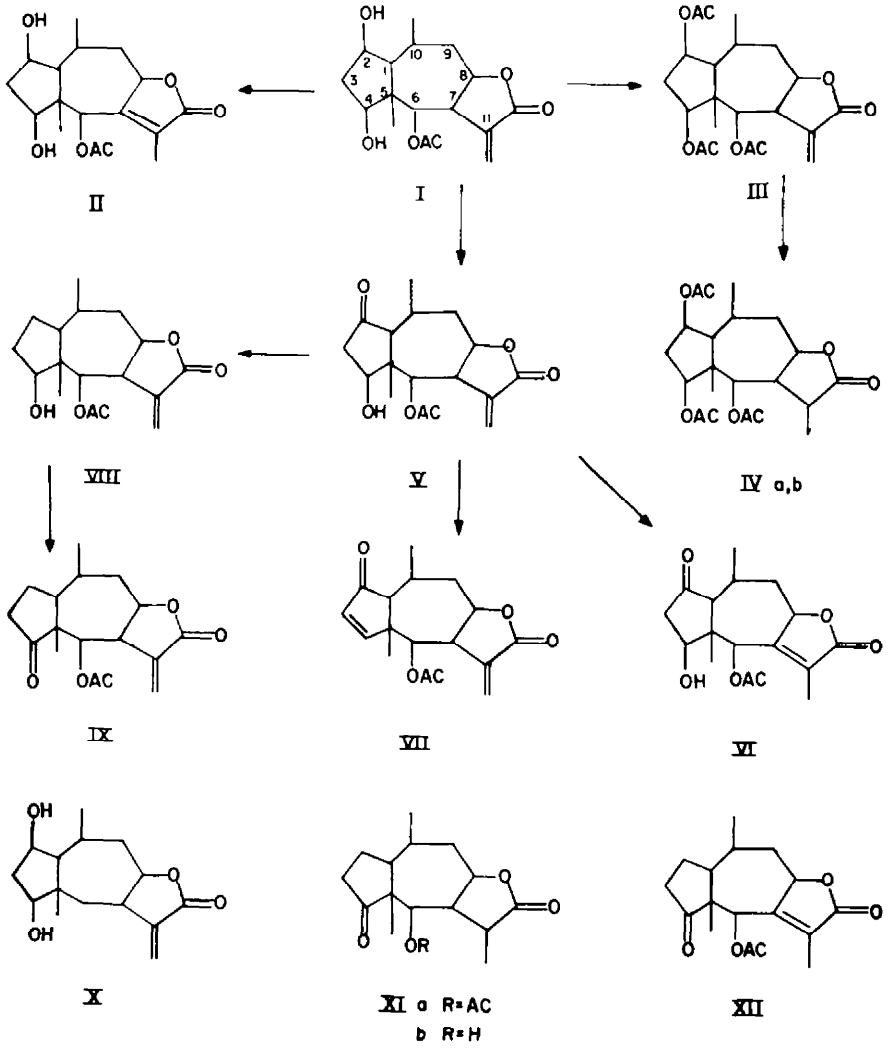
The presence of partial structure C is demonstrated by the following transformations. Partial oxidation of I yields, as in the case of pulchellin (X),[4b] dehydroflexuosin A (V) which is clearly a cyclopentanone derivative (new carbonyl bond superimposed on acetate absorption at 1740 cm^{-1}). The NMR spectrum indicates that oxidation of the hydroxyl group responsible for the complex multiplet near 4·16 ppm has taken place. Treatment of V with methanesulfonyl chloride results in dehydration and formation of an α,β-unsaturated cyclopentenone, (VII), λ_{max} 218 and 320 mμ (ε 15100 and 32), IR bands at 1760, 1745, 1710 and 1660, whose NMR spectrum has two new vinyl doublets at 7·71 d (J = 6) and 6·02 d (J = 6). The lack of further splitting requires complete substitution of the γ-position (C_5) which in conjunction with the multiplicity of H_2 in the NMR spectrum of I leads to expression C. Incidentally, the formation of VII also demonstrates that while H_4 is adjacent to two protons at C_3, one of the coupling constants is close to zero.

Consideration of partial structures A and C and the NMR spectra now rule out

[5] Multiplicities are indicated by the usual symbols d doublet, t triplet, q quartet, c complex multiplet whose center is given, br unresolved doublet whose half-height width is given. Spectra were run in deuterochloroform with tetramethylsilane as internal standard.

[6] The triacetate III is the sole product on treatment of flexuosin A with sodium acetate–acetic anhydride. Reaction of I with isopropenyl acetate furnishes the diacetate i as well as III.





definitely the presence of a second, hindered double bond. Flexuosin being therefore bicyclic, the necessity for attaching partial structure A to the second ring together with the presence of a secondary and a tertiary methyl group requires fusion of a seven-membered ring to C_1 and C_5, (through R_1 and R_2) with R_3 being replaced by the tertiary methyl group. Now in all NMR spectra of flexuosin derivatives, hydrogen on carbon carrying the acetate function is found consistently as a clean doublet. This requires its position at C_6 and simultaneously, the attachment of either the lactone side chain or the secondary methyl group to C_7.

The former is of course overwhelmingly more probable on biogenetic grounds and would lead directly to formula I. More concrete evidence is furnished by inspection of the NMR spectrum of isoflexuosin A. The signal of H_6, now no longer a doublet, appears at the unusually low field of 6·36 ppm. These facts are consonant with its allylic character required by formula II for isoflexuosin A and hence I for its

precursor and rule out alternative proposals for placing the lactone ring elsewhere. Independent evidence for the attachment of the lactone side chain to C_7 through dehydrogenation of flexuosin A could not be adduced (Experimental).

Attempts have been made to correlate flexuosin A with other constituents of *Helenium* species. Conversion of V to the thioketal followed by desulfurization yields gummy 2-desoxyflexuosin A (VIII). The latter on chromic acid oxidation furnishes a crystalline substance (IX), m.p. 155–158°, $[\alpha]_D^{24}$ +35·7°, apparently isomeric with 2,3-dihydrobigelovin.[7] Unfortunately, lack of material prevented reduction of IX by catalytic or chemical means to one of the several isomers of dihydroisotenulin (XIa) of established stereochemistry[2] although by analogy with the results described for flexuosin itself (*vide supra*) isomerization to a substance XII rather than hydrogenation was envisioned as a distinct possibility. Such an isomerization (to VI) occurred also in the course of an attempt to hydrogenate V with platinum oxide in acetic acid. In fact the only crystalline "normal" reduction products, (IVa and IVb, presumably C_{11}-epimers) are obtained by catalytic hydrogenation of III, using palladium-charcoal in ethanol.

Failure of the catalytic method in the case of flexuosin A prompted a study of its reaction with sodium amalgam. This results not only in reduction of the exocyclic methylene group, but hydrolysis of the acetate function. The product was oxidized to a diketone (IR bands at 3500-OH, 1775-γ-lactone, 1745-cyclopentanone—and 1713 cm^{-1}-cycloheptanone) which was converted to a crystalline mesylate. Treatment of the latter with sodium acetate results in elimination and formation of a cyclopentenone. Because the NMR spectra of these compounds exhibit a low-field doublet near 5 ppm characteristic of hydrogen on carbon carrying lactone ether oxygen instead of the more complex signal found in I–IX we assume that the sodium-amalgam treatment results in lactone ring reorientation and ascribe structures XIII, XIV and XV to this series of compounds (the reliability of this criterion has been tested repeatedly in the helenalin, tenulin and balduilin series).

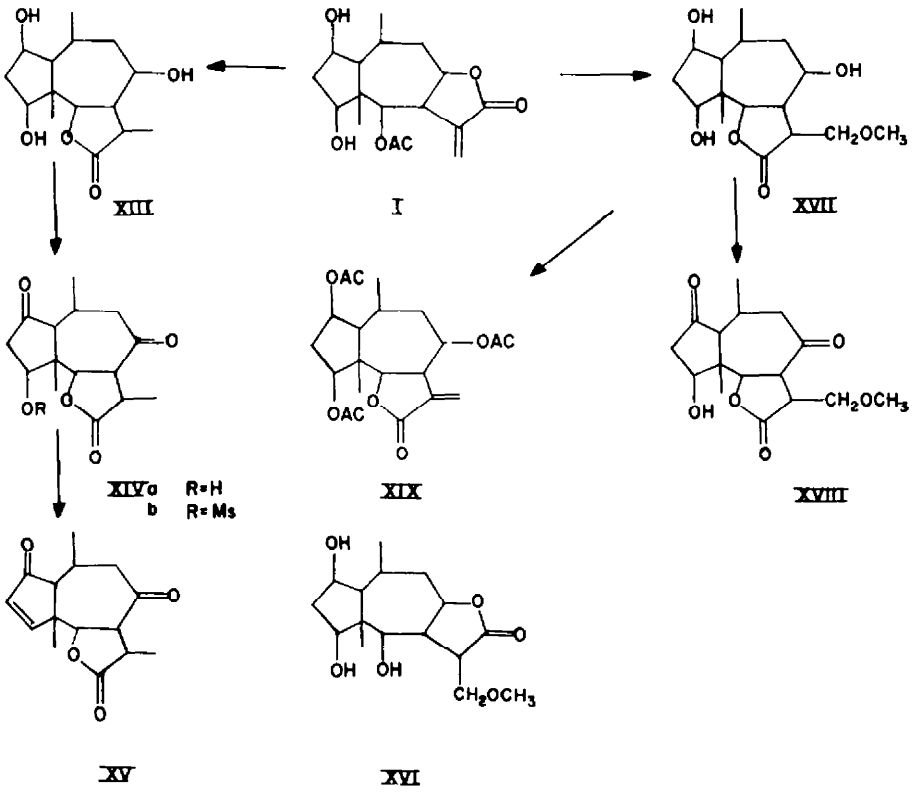
Treatment of flexuosin A with methanolic potassium carbonate which proved effective for hydrolyzing dihydroisotenulin without lactone reorientation[8] furnishes a methoxy derivative XVI or XVII.[4b,9] Oxidation of the latter results in a hydroxy-diketolactone (relevant IR bands at 1780—γ-lactone, 1748—cyclopentanone—and 1705 cm^{-1}—cycloheptanone) whose NMR spectrum exhibits a sharp doublet at 5·50 d (J = 10·5) which must be ascribed to hydrogen on carbon carrying the lactone ether oxygen since H_4 is generally found farther upfield (cf. the NMR spectrum of XIVa). We therefore assign formulae XVII and XVIII to these compounds and structure XIX to an α,β-unsaturated lactone obtained from XVII by treatment with sodium acetate–acetic anhydride. The latter which clearly differs from III must be the diacetate of an isomer of I with lactone ring reorientated, i.e. the diacetate of a substance which we name alloflexuosin A in accordance with established procedure.

Flexuosin B, previously[3] assigned formula $C_{17}H_{24}O_6$, m.p. 132–137°, is actually a solvate $C_{20}H_{28}O_6 \cdot H_2O$ of variable melting point. Its purity was attested by its

[7] B. A. Parker and T. A. Geissman, *J. Org. Chem.* **27**, 4127 (1962).

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

[9] G. H. Kulkarni, A. Paul, A. S. Rao, G. R. Kelkar and S. C. Bhattacharya, *Tetrahedron*, **12**, 178 (1961).



thin-layer chromatographic behavior and the formula established by analyses of several derivatives and proton counts of their NMR spectra.

The IR spectrum (nujol) clearly establishes the presence of an hydroxyl group, a γ-lactone (1765 cm^{-1}) and a double bond (1648 cm^{-1}) which is conjugated (UV absorption at 219·5 and 300 mμ, ε 11900 and 27), with the γ-lactone or one of two other carbonyl groups (bands at 1748 and 1710 cm^{-1}), one of which is probably an ester (bands at 1240 and 1220 cm^{-1}). That the unsaturation is not associated with the functional groups previously found in constituents of *Helenium* species[10] was established by ozonolysis which liberates 60% of acetone.

This result, in conjunction with the empirical formula, suggests that flexuosin B is the dimethylacrylate of a sesquiterpene lactone $C_{15}H_{22}O_5$ and that the UV spectrum results from the presence of this chromophore,[11] responsible for the IR bands at 1710 and 1660 cm^{-1} and that of a cyclopentanone, responsible for the band at 1748 cm^{-1}. The NMR spectrum which has a slightly split vinyl proton resonance at 5·62 ppm and two vinyl methyl doublets (J = 1) at 2·16 and 1·89 ppm provides strong support for this hypothesis;[12] other signals occur at 5·45 br (hydrogen on carbon carrying ester function), 4·71 c (hydrogen on carbon carrying lactone ether oxygen) 4·3 c

[10] W. Herz, *J. Org. Chem.* **27**, 4043 (1962).

[11] A. T. Nielsen, *J. Org. Chem.* **22**, 1539 (1957).

[12] cf. the NMR spectrum of *p*-bromophenacyl dimethylacrylate which has signals at 5·76, 2·20 and 1·92 p.p.m.

(hydrogen on carbon carrying hydroxyl), 1·48 d (7) and 1·27 d (5, two secondary methyls) and 0·88 ppm (tertiary methyl group).

The transformations shown below establish the relationship of the functional groups and require expression XX for flexuosin B, exclusive of stereochemistry. Catalytic hydrogenation of XX consumes one mole-equivalent of hydrogen and yields XXI whose NMR spectrum indicates the disappearance of the vinyl proton and the presence of two additional secondary methyl groups. Dehydration of XX or XXI affords the cyclopentenones XXII (relevant IR bands at 1775, 1717, 1650 and 1575 cm^{-1}) and XXIII (λ_{max} 227 and 318 mμ, ε 19400 and 46,) IR bands at 1775 1745, 1715 and 1575 cm^{-1}), both of which exhibit the typical NMR signals of α,β-unsubstituted-γ-monosubstituted cyclopentanones.[4a,8,10] Hence the hydroxyl group of flexuosin B is secondary and β- to the cyclopentanone carbonyl.

Catalytic reduction of XXII and XXIII furnishes the same cyclopentanone derivative XXIV. Hydrolysis of XXIV with methanolic potassium hydroxide at room temperature affords isovaleric acid and dihydromexicanin C (XXV) of established structure and stereochemistry.[4b,13] That no lactone ring reorientation has accompanied the hydrolysis is clearly demonstrated by the NMR spectra of XXII and XXIII which exhibit the usual complex triplet of H_8 considerably upfield from the unresolved doublet of H_6 near 5·5 ppm. Hence the lactone ring of flexuosin B is closed to C_8 and the only remaining question is whether the C_{11}-methyl group of flexuosin B possesses the configuration of the C_{11}-methyl group of tetrahydrohelenalin (XXVI) or of dihydromexicanin C, i.e. whether the conversion of XXIV to XXV is accompanied by inversion at C_{11} or not.

This matter was easily settled by synthesis. Reaction of tetrahydrohelenalin with isovaleroyl chloride yields a compound identical in all respects with tetrahydroanhydro flexuosin B. Hence the complete structure and stereochemistry of flexuosin B is given by formula XX where the only uncertainty is the configuration of the hydroxyl group at C_2.

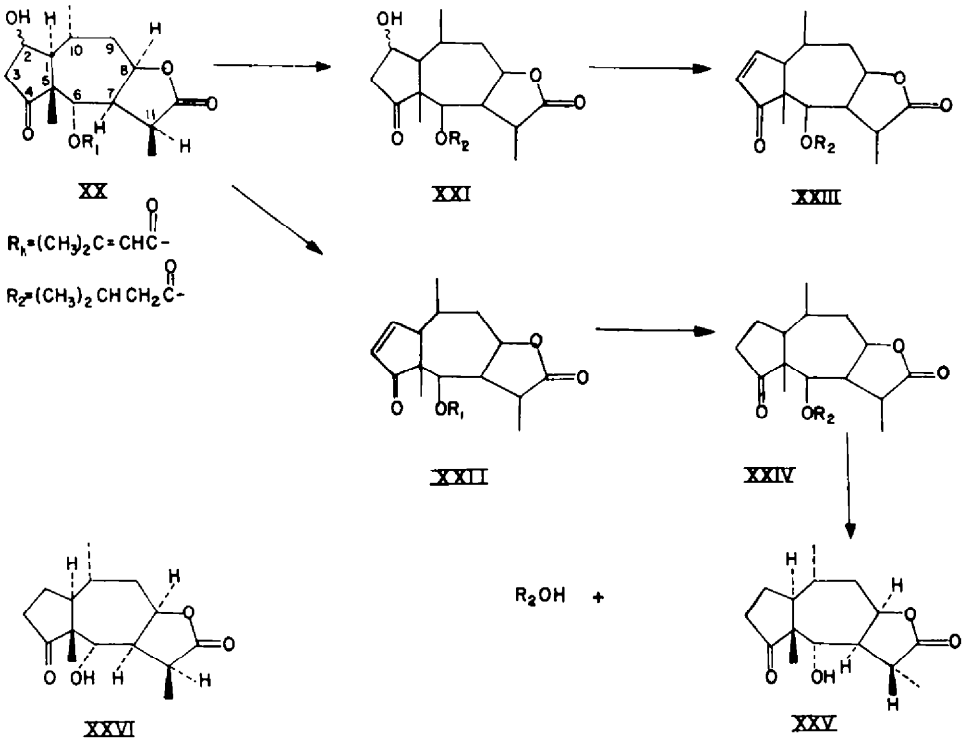
EXPERIMENTAL[14]

Flexuosin A(I). The material used was mainly that isolated previously.[3] An attempt was made to obtain additional flexuosin A and B from various collections of *H. flexuosum* Raf. made by Drs H. F. L. Rock and P. Redfearn in Tennessee and Missouri during July and August 1960 at the flowering stage. These were combined and extracted in the usual fashion. However, this resulted in large quantities of non-crystallizable gums and very small amounts of flexuosin A and B were isolated only after laborious chromatography. It is not known whether this resulted from the combination of collections representing different races or different stages in the growth cycle.

Flexuosin A consumed 1 mole of perbenzoic acid after 5 days in the refrigerator. A solution of 0·2 g flexuosin A in 15 ml acetic acid was ozonized at 0° until ozone was no longer absorbed (1 hr). Zinc dust and water were added to the solution which was distilled into a solution of 0·15 g dinitrophenylhydrazine in 50 ml 2 N HCl, yield of formaldehyde dinitrophenylhydrazone, m.p. 162·5–163·5°, 0·05 g (39%). Recrystallization raised the m.p. to 163–164°, undepressed on admixture of an authentic sample.

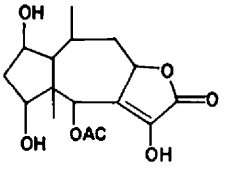
[13] Since the absolute configuration of these compounds has not been determined, the actual structures may be mirror images of the formulae given in this paper although the ORD curves suggested that they are correct as written.[3] (See note added at the end of the paper.)

[14] M.ps and b.ps are uncorrected. Analyses by Dr. F. Pascher, Bonn, Germany. IR spectra were run in chloroform, rotation in 95% ethanol unless otherwise specified. NMR spectra were run in A-60 or HR-60 instruments in deuteriochloroform solution, tetramethylsilane serving as internal standard. The A-60 spectrometer was purchased with the aid of a grant from the National Science Foundation.



Ozonolysis of flexuosin A in methanol at −70° in the manner described for pulchellin[4a] gave a non-crystallizable enolic gum, presumably XXVII, which did not furnish a crystalline acetate or benzoate, λ_{max} 219·5, 277 and 325 mμ (ε 9260, 685, 180), strong positive ferric chloride test.

Flexuosin was recovered when allowed to stand with acetone and perchloric acid and was unchanged on refluxing with methanolic H_2SO_4 or HCl.



XXVII

Diacetylflexuosin A(III). A mixture of 0·5 g flexuosin A, 0·5 g sodium acetate and 3 ml acetic anhydride was refluxed for 8·5 hr, poured into ice-water, digested overnight and extracted with methylene chloride. The extract was washed, dried and evaporated and the gummy residue chromatographed over a 1·5 × 15 cm column of neutralized alumina.[15] Benzene and benzene–chloroform (3:2) eluted prisms, wt. 0·11 g, m.p. 129° which on recrystallization m.p. 129–130°, $[\alpha]_D^{24}$ −11·4° [*c*, 0·79], IR band, ($CHCl_3$) 1750 and 1725 cm^{-1}, (KBr pellet) 1770 (γ-lactone), 1730 and 1240 (ester) and 1660 cm^{-1} (double bond), UV end absorption, NMR signals at 6·14 d(3) and 5·42 d (3)—exocyclic methylene, 5·79 d (5)—H_6, 5·02, td (7·5, 3)—H_8, 4·77 d (5)—H_4, 2·12, 2·03, 2·00 (3 acetate methyls), 1·05 d (6), C_{10}-methyl) and 0·85 ppm (C_4-methyl). (Found: C, 61·36; H, 7·03, O, 31·59. Calc. for $C_{21}H_{28}O_8$: C, 61·75; H, 6·91; O, 31·34%).

Monoacetylflexuosin A. A mixture of 1 g flexuosin A, 0·15 g *p*-toluenesulfonic acid monohydrate

[15] Alcoa F-20 alumina was washed with 2·5% HCl water and distilled water until no positive test was obtained with $AgNO_3$ aq., washed with methanol, dried in air at room temp and reactivated by heating for 7 hr at 250°.

and 20 ml isopropenyl acetate was refluxed with a Stark and Dean trap for 16 hr, cooled and extracted with ether. The extract was washed, dried and evaporated and the residue chromatographed over neutralized alumina (2 × 13·5 cm). Benzene and benzene-chloroform (5:1 and 1:1) eluted solid material, m.p. 126–133°. Repeated recrystallization from ethyl acetate–hexane furnished 0·2 g diacetylflexuosin A, m.p. 129–130°. The mother liquors deposited fine colorless plates in a separate colony, m.p. 154–158°, mixed m.p. with diacetylflexuosin A 114–120°. Further recrystallization raised the m.p. to 158–160°, IR bands (KBr pellet) at 3450, 1765 (γ-lactone), 1745 and 1245 (acetate) and 1650 cm^{-1} (double bond). (Found: C, 62·35; H, 7·06. Calc. for $C_{19}H_{26}O_7$: C, 62·28; H, 7·15%).

Isoflexuosin A(II). A solution of 0·542 g flexuosin A in 30 ml acetic acid was reduced (0·05 g prereduced PtO_2 at atm. press., H_2 uptake, found 27·3 ml, calc. 39 ml). The solution was filtered, concentrated and the residual oil chromatographed over 25 g acid washed alumina. Chloroform eluted colorless needles, wt. 0·161 g, which were recrystallized from acetone and a small amount of hexane, m.p. 219–221°, mixed m.p. with flexuosin A 209–213°, λ_{max} 223 mμ (ε 14800), IR bands at 3500, 1755 (γ-lactone), 1725 (acetone) and 1660 cm^{-1} (double bond), NMR signals at 6·36 (H_6, broadened somewhat by long-range coupling), 5·3 c (H_8), 4·18 br and 3·8 c (H_3 and H_4), 2·23 (acetate methyl), 1·83 (slightly split, vinylic C_{11}-methyl), 1·23 d (5,C_{10}-methyl) and 0·92 ppm (C_4-methyl). (Found: C, 62·81; H, 7·68; O, 29·85. Calc. for $C_{17}H_{24}O_6$: C, 62·95; H, 7·46; O, 29·60%).

The remaining gummy fractions were obviously mixtures of dihydro-derivatives. Oxidation of 0·2 g of this material with chromic oxide–pyridine furnished a gum which did not crystallize after extensive chromatography. The IR spectrum exhibited an intense new band at 1742 cm^{-1} characteristic of cyclopentanones.

Hydrogenation of flexuosin A with Pd–C in ethanol in a Parr hydrogenator or at atm. press. failed to give crystalline material even after extensive chromatography. The combined gums from one such reduction utilizing 0·6 g flexuosin A were reduced with $LiAlH_4$ in boiling tetrahydrofuran. Work-up in the usual fashion furnished a glass which was dehydrogenated with Pd–C. The charred mass was extracted with hexane (slight bluish green color), the azulenes taken up in 70% phosphoric acid, the acid extract diluted with ice-water and extracted with hexane. Concentration (red. press.) resulted in a small amount of bluish oil which did not furnish a crystalline trinitrobenzene complex. The material was decomposed by chromatography over alumina. Thin-layer chromatography established the presence of several azulenes, but it was not possible to identify these definitely as guaiazulene, chamazulene, artemazulene, linderazulene or lower homologs thereof.

Dihydrodiacetylflexuosin A (IVa and b). A solution of 0·534 g III in 15 ml ethanol was hydrogenated with 0·05 g Pd-C at atm. press. until H_2 uptake ceased (found 27 ml, calc. 29 ml), filtered and evaporated (red. press.) The gummy residue, wt. 0·513 g, was chromatographed over acid-washed alumina (1·5 × 20 cm). Benzene–chloroform (1:1) eluted two fractions (0·397 and 0·11 g) of colorless gum which partially crystallized on trituration with ether. Recrystallization of the first fraction from acetone–ether–hexane furnished needles (IVa), m.p. 135–138°, $[\alpha]_D^{24}$ +64·2° (*c*, 0·98). The IR spectrum had bands (CCl_4) at 1791 (γ-lactone) and 1754 cm^{-1} (acetate), NMR signals at 5·42 br (H_6) 4·8 d (6, H_4) superimposed on complex signals of H_3 and H_8—total intensity 3 protons, 2·10 (6 protons) and 2·03 (3 protons, acetate methyls), 1·20 d (7) and 1·02 d (6, C_{10}- and C_{11}-methyls) and 0·80 ppm. (C_5-methyl). (Found: C, 61·37; H, 7·50; O, 31·38. Calc. for $C_{21}H_{30}O_8$: C,61·45; H, 7·37; O, 31·18%).

Recrystallization of the second fraction from acetone–ether–hexane gave what was presumably the C_{11}-epimer (IVb) of IVa, m.p. 148–150°, $[\alpha]_D^{24}$ −36·1° (*c*, 1·6). Its IR spectrum was practically indistinguishable from that of IVa. (Found: C, 61·10; H, 7·43. Calc. for $C_{21}H_{30}O_8$: C, 61·45; H, 7·37%).

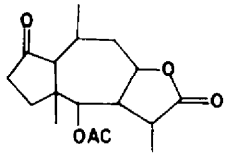
Dehydroflexuosin A (V). A solution of 0·3 g flexuosin A in 3 ml pyridine was added with stirring to the complex prepared from 1 g chromium oxide and 10 g pyridine, stirred for 2 hr and kept at room temp. overnight. Excess oxidant was decomposed with ethanol, the mixture diluted with water and thoroughly extracted with ether. The dried ether extracts were concentrated and the solid residue recrystallized from ethyl acetate–hexane, m.p. 185°, $[\alpha]_D^{24}$ +75·5° (*c*, 0·89) IR bands at 3580, 1755 (γ-lactone), 1740 (double strength-combination of cyclopentanone and acetate) and 1670 cm^{-1} (double bond), NMR signals ($CDCl_3$ and a little D_2O) at 6·15 d (3) and 5·43 d (3, exocyclic methylene), 6·17 d (5, H_6), 4·60 td (11, 3, H_8), 3·58 d (5,H_4), 2·05 (acetate methyl), 1·40 d (6,C_{10}-methyl) and 0·885 p.p.m. (C_5-methyl). (Found: C, 63·29; H, 6·99; O, 30·21. Calc. for $C_{17}H_{22}O_6$: C, 63·34; H, 6·88; O, 29·78%).

The dinitrophenylhyrdazone melted at 247° (dec), λ_{max} ($CHCl_3$) 294 and 363 mμ (ε 2760 and 26450). (Found: C, 55·34; H, 5·12; N, 10·85. Calc. for $C_{22}H_{26}O_9N_4$: C, 54·97; H, 5·22; N, 11·55%).

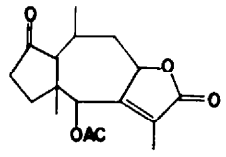
Dehydroisoflexuosin A (VI). A solution of 0·152 g V in 16 ml acetic acid was hydrogenated with 0·015 g prereduced PtO_2 at atm. press., H_2 uptake 10·1 ml, calc. 11·5 ml. Concentration furnished a gum which was chromatographed over acid-washed alumina (1·5 × 20 cm). The fractions eluted with benzene–chloroform (1:1) gave needles, m.p. 169–170°, yield 5 mg, λ_{max} 222·5 mμ (ε14100), IR bands (KBr pellet) at 3500, 1750, 1720 and 1670 cm^{-1}. (Found: C, 63·36; H, 6·88; O, 29·68. Calc. for $C_{17}H_{22}O_6$: C, 63·34; H, 6·88; O, 29·78%).

Anhydrodehydroflexuosin A (VII). To a cold solution of 0·256 g VI in 2 ml pyridine was added with stirring 0·2 g methanesulfonyl chloride. After keeping overnight at room temp., the solution was poured over ice and extracted with methylene chloride. The extract was washed, dried and concentrated and the gummy residue recrystallized from aqueous methanol, m.p. 149–150°. The IR spectrum of this sample indicated the presence of methanol, of crystallization. After drying at room temp. over P_2O_5 *in vacuo* for 3 days it melted at 135–139°, yield 0·178 g., $[\alpha]_D^{24}$ +56·8° (*c*, 0·85), IR spectrum at 1760 (γ-lactone), 1745 (shoulder, acetate), 1710 (cyclopentenone) and 1660 cm^{-1} (double bond), λ_{max} 218 and 320 mμ (ε 15100 and 32), NMR signals at 7·71 d(6) and 6·02 d (6, H_3 and H_4), 6·33 d (3·5) and 5·49 d (3·5, exocyclic methylene), 5·76 d (4·5, H_6), 4·84 d (H_5), 2·08 (acetate methyl), 1·47 d (4, C_{10}-methyl) and 1·07 ppm (C_5-methyl). (Found: C, 66·94; H, 6·62; O, 26·59. Calc. for $C_{17}H_{24}O_5$: C. 67·09; H, 6·62; O, 26·29%).

Hydrogenation of this material in methanol with prereduced Pd-C at atm. press. consumed 1·4 moles H_2. The product could not be crystallized even after extensive chromatography. Its IR (bands at 1784, 1770, 1745 and 1660 cm^{-1}) and UV spectra (λ_{max} 219 mμ, ε 5110) indicated the presence of a mixture of XXVIII and XXIX.



XXVIII



XXIX

Dehydrodesoxydehydroflexuosin A (IX). A mixture of 0·34 g dehydroflexuosin A, 30 ml dry benzene, 1 ml boron trifluoride etherate and 1·3 ml ethanedithiol was kept at room temp. for 15 hr, diluted with water, made alkaline ($NaHCO_3$ aq.) and extracted with ether. The extract was washed, the washings reextracted with methylene chloride and the combined organic extracts dried and concentrated. The gummy residue which had IR bands at 3500, 1770 and 1740 cm^{-1} (shoulder) was taken up in 200 ml absolute ethanol, refluxed with 13 g freshly prepared Raney nickel for 16 hr, filtered and concentrated, yield of gummy product (VIII) 0·252 g, IR bands (CCl_4) at 3500, 1680(γ-lactone), 1745 and 1238 cm^{-1} (acetate). It was dissolved in 5 ml dry pyridine and oxidized with 0·5 g chromic oxide in 5 ml pyridine in the same manner as flexuosin A. The usual work up furnished a brown gum which was chromatographed over acid-washed alumina (1·5 × 21 cm). The fractions eluted with benzene–chloroform (1:1) crystallized on trituration with ether. Recrystallization from ether hexane furnished 0·03 g IX, m.p. 155–158°, $[\alpha]_D^{24}$ +35·7° (*c*, 0·65), positive Brady and Zimmermann test, IR bands at 1765 (γ-lactone), 1750 (double strength-cyclopentanone and acetate) and 1402 cm^{-1} ($-CH_2-\overset{\overset{O}{\|}}{C}-$), λ_{max} 213 and 292 mμ. (Found: C, 66·13 H, 7·71; O, 26·11. Calc. for $C_{17}H_{24}O_5$: C, 66·21 H, 7·85, O, 25·94%).

Desacetyldihydroalloflexuosin A (XIII). A mixture of 1 g flexuosin A, 25 g 3% NaHg and 75 ml 95% ethanol was stirred at room temp. for 5 hr and then at 50–55° for 3 hr. The solution was cooled, acidified with dil. HCl and concentrated to dryness (red. press.) The residue was extracted repeatedly with boiling acetone and the extract concentrated to 20 ml. 0·5 g of colorless prisms, separated which melted at 216–217° after recrystallization from acetone and a little hexane, $[\alpha]_{24}^{D}$ −54·5° (*c*, 1·4), no UV absorption, IR bands (nujol) at 3570, 3400 and 1755 cm^{-1}. The NMR spectrum was difficult

to determine due to insolubility but exhibited (dimethyl-sulfoxide) a complex series of bands corresponding to 4 protons in the range 5–5·8 ppm, 2 methyl doublets at 1·25° d (J = 6) and 1·03 d (J = 6) and a methyl singlet at 0·61 ppm. (Found: C, 63·50, H, 8·37; O, 28·30. Calc. for $C_{15}H_{24}O_5$: C, 63·36; H, 8·51; O, 28·14%).

Bis-dehydrodesacetyl dihydroalloflexuosin A (XIVa). A solution of 0·478 g XIII in 10 g pyridine was added to a stirred suspension of pyridine–chromium trioxide complex prepared from 1·2 g chromium trioxide and 10 g pyridine. After 12 hr at room temp. with stirring, ethanol was added to destroy excess oxidizing agent. The mixture was diluted with water, neutralized with dilute acid and extracted thoroughly (ether and methylene chloride). Both extracts were separately washed, dried and evaporated, but the residues proved to be the same substance. They were combined and chromatographed over acid-washed alumina (1·5 × 21 cm). Benzene–chloroform (1:1), chloroform and chloroform–methanol (20:1) eluted 0·333 g solid, m.p. 190–196°. Recrystallization from acetone–hexane furnished prisms, m.p. 195–196·5°, $[\alpha]_D^{24}$ −56·8° (*c*, 1·77), IR bands at 3500 (broad, OH), 1775 (γ-lactone), 1745 (cyclopentanone) and 1713 cm^{-1} (cycloheptanone), NMR signals at 5·60 d (10, H_8), 4·30 d (5, H_4), 3·4 c (2 protons), 2·6 c (6 protons), 1·37 d (5) and 1·33 d (7, C_{10}- and C_{11}-methyls) and 0·67 ppm (C_5-methyl). (Found: C, 64·09; H, 7·37; O, 28·83. Calc. for $C_{15}H_{20}O_5$: C, 64·27; H, 7·19; O, 28·54%).

Dehydration of XIVa. A mixture of 0·066 g XVIa, 1 ml pyridine and 0·066 g methanesulfonyl chloride was allowed to stand at room temp for 20 min, decomposed with ice water, filtered and the precipitate recrystallized from acetone–methanol. The product (XIVb) m.p. 154–155°, wt. 0·029 g had IR bands at 1780 (γ-lactone), 1750 (cyclopentanone), 1705 (cycloheptanone), 1363, 1168 and 1160 cm^{-1} (mesylate). The mother liquors contained a mixture of XIVb and XV. (Found: C, 53·24; H, 6·30, O, 31·53; S, 9·19. Calc, for $C_{16}H_{22}O_7S$: C, 53·61; H, 6·19; O, 31·25; S, 8·95%).

A solution of 0·187 g XIVb and 0·204 g sodium acetate in 25 ml ethanol was refluxed 1 hr, concentrated to dryness *in vacuo* and the residue extracted thoroughly with methylene chloride. The extract was washed, dried and evaporated and the residue chromatographed over alumina (1·5 × 10 cm^{-1}). Benzene and benzene–chloroform (1:1) eluted needles (XV), which were recrystallized from acetone–hexane, first crop wt. 0·04 g, m.p. 187–190°, second crop wt. 0·02 g m.p. 173–185°. Further recrystallization raised the m.p. to 191–193°, $[\alpha]_D^{24}$ −88·6° (*c*, 1·16), λ_{max} 218 and 307 mμ (ε 8920 and 26), IR bands at 1780 (γ-lactone), 1710 (cyclopentenone and cycloheptanone), and 1600 cm^{-1} (double bond); NMR signals at 7·70 d (6) and 6·14 d (6, H_3 and H_4), 4·88 d (7, somewhat broadened by long-range splitting, H_6), 1·50 d and 1·32 d (6, C_{10}- and C_{11}-methyls) and 0·87 ppm (C_5-methyl). (Found: C, 68·40; H, 7·08; O, 24·87. Calc. for $C_{15}H_{18}O_4$: C, 68·68; H, 6·92; O, 24·40%).

Preparation of XVII *and its oxidation.* A solution of 0·3 g flexuosin A, 0·6 g K_2CO_3, 72 ml methanol and 18 ml water was kept at room temp. for 48 hr, neutralized with acetic acid and evaporated to dryness *in vacuo*. The residue was extracted with ether and methylene chloride, the organic extracts were combined and evaporated. The residue gradually solidified. Recrystallization from ethyl acetate–hexane afforded XVII, m.p. 150–152°, wt. 0·12 g, $[\alpha]_D^{24}$ −57·7°, IR bands (Br) 3500, 3350 and 1760 cm^{-1}. (Found: C, 61·38; H, 8·13; O, 30·49. Calc. for $C_{16}H_{26}O_6$: C, 61·13, H, 8·34; O, 30·54%).

Oxidation of 0·8 g XVII with chromic acid–pyridine was carried out as described for XIII. The usual work-up furnished a brown gum which was dissolved in a small amount of benzene and chromatographed over acid-washed alumina (1·5 × 13·5 cm^{-1}). The fractions eluted with benzene–chloroform (1:1) and chloroform were combined and rechromatographed. Chloroform then eluted a gum which on treatment with ether gradually deposited pale yellow prisms (XVIII), wt. 0·285 g, m.p. 133° $[\alpha]_D^{24}$ −73·5 (*c*, 0·872), IR bands at 3610 and 3400 (—OH), 1780 (γ-lactone), 1748 (cyclopentanone) and 1705 cm^{-1} (cycloheptanone), NMR signals at 5·50 d (10·5, H_6), complex series of bands from 3·5–4·3 ppm due to superposition of —CH_2—O— and H_4, 3·32 (methoxyl), 1·35 d (5, C_{10}-methyl) and 0·58 ppm (C_5-methyl). XVIII could also be prepared by chromic acid–sulfuric acid oxidation of XVII, but in somewhat lower yield (0·121 g from 0·656 g XVII). (Found: C, 61·87; H, 7·10; O, 30·79. Calc. for $C_{16}H_{22}O_6$: C, 61·92; H, 7·15; O, 30·93%).

Diacetylflexuosin A (XIX). A mixture of 0·18 g XVII, 0·206 g anhydrous sodium acetate and 3 ml acetic anhydride was refluxed 8 hr, cooled, poured over ice-water, digested overnight and thoroughly extracted with methylene chloride. The usual work-up furnished a gum which was chromatographed over acid washed alumina (23 g). One of the fractions eluted with benzene–chloroform (1:1) afforded solid material which was recrystallized from ethyl acetate–hexane, yield 0·1 g, m.p. 176 177°, $[\alpha]_D^{24}$ − 31° (*c*, 1·03), λ_{max} 211 mμ (ε 9900), IR bands at 1770 (γ-lactone) 1745 and 1235 (strong, acetates),

and 1660 cm^{-1} (double bond), NMR signals at 6·02 d (4) and 5·53 d (4, exocyclic methylene group), 5 c (4 protons, H_2,H_4, H_6 and H_8), 0·94 d (C_{10}-methyl) and 0·70 ppm (C_5-methyl). (Found: C, 61·57; H, 7·23; O, 31·02; CH_3CO, 31·62. Calc. for $C_{21}H_{28}O_6$: V, 61·75; H, 6·91; O, 31·34; CH_3CO, 31·62%).

Flexuosin B (XX). The material used was largely that isolated previously[3] although small additional quantities were obtained as outlined in the section on flexuosin A. The m.p., previously described as 132°–137°, proved to be exceedingly variable due to solvation and is no criterion of purity. Material recrystallized from dil. methanol now melted at 107–110°, but did not clear completely till 123°, positive Zimmermann test, positive test with Brady's reagent (slow), negative periodate and ferric chloride test, IR bands at 3650, 3600, 3450 (OH), 1770 (γ-lactone), 1745 (cyclopentanone), 1710 (conjugated ester) and 1645 cm^{-1} (double bond) (nujol), 3510, 3400, 3250, 1765, 1748, 1700, 1648, 1240 and 1220 cm^{-1}, λ_{max} 219·5 and 300 mμ, (ε 11900 and 30), NMR signals at 5·56 (slightly split, H_6 or vinyl hydrogen), 5·45 (slightly split), vinyl hydrogen or H_8), 4·7 c (H_8), 4·3 c (H_3), 2·16 d (1) and 1·89 d (1, vinyl methyls), 1·48 d (7) and 1·27 d (5, C_{10}- and C_{11}-methyl) 0·882 ppm (C_5-methyl). For analysis the sample was dried at room temp. *in vacuo* for 3 days over P_2O_5. (Found: C, 63·12; H, 7·94; O, 28·82. Calc. for $C_{20}H_{28}O_6.H_2O$: C, 62·81; H, 7·91; O, 29·29%).

A sample of m.p. 124–133° was obtained by recrystallizing flexuosin B from aqueous ethanol or acetone. The m.p. was changed to 110–125° by drying this material over P_2O_5 for 3 days in a high vacuum. All 3 samples gave a single spot and were indistinguishable from each other on thin layer chromatography using four solvents (R_f 0·12 3:1 benzene–ether, R_f 0·30 1:1 benzene ether, R_f 0·45 1:3 benzene–ether, R_f 0·67 ether). IR spectra were superimposable.

Ozonolysis of flexuosin B. A solution of 0·297 g flexuosin B in 20 ml acetic acid was ozonized at 5° until ozone was no longer absorbed. Zinc dust and water was added and the mixture was slowly distilled into a solution of dinitrophenylhydrazine in 2 N HCl. Yellow needles were formed, filtered, dissolved in benzene and chromatographed over acid washed alumina, (1·5 × 15·5 cm [1]). Benzene eluted 0·11 g (60%) acetone dinitrophenylhydrazone, mixed m.p. undepressed.

The oxonolysis was repeated and the mixture steam distilled into a dimedone solution. No formaldehyde could be detected.

Dihydroflexuosin B. (XXI). A solution of 0·87 g flexuosin B (m.p. 122–135°) in 30 ml ethanol was reduced with 0·5 g 5% Pd-C at atm. press., observed uptake 53 ml (corr) calc for 2 double bonds 51 ml. The solution was filtered, concentrated (red. press) and chromatographed over acid-washed alumina (1·5 × 20 cm). The fractions eluted with benzene–chloroform (1:1) and chloroform, wt. 0·8 g, solidified on trituration with a small amount of ether, m.p. 82–87°. Reduction in ethyl acetate solution with prereduced Pd-$CaCO_3$ gave the same material, m.p. 85°, $[\alpha]_D^{24}$ +43·9°(*c*, 1·14), IR bands at 3660, 3600, 3460 (—OH), 1775 (γ-lactone), 1750 (cyclopentanone) 1725 (ester), (nujol) 3600, 3400, 3300, 1760, 1745, 1725, 1192 cm^{-1}. The UV spectrum, (λ_{max} 225 mμ and 276 mμ ε 700 and 60) indicated contamination by a small amount of XXIII (*vide infra*). The sample stubbornly retained water, but analyzed correctly after prolonged drying at room temp. (Found: C, 64·63; H, 8·27; O, 27·23. Calc. for $C_{20}H_{30}O_6$. 1/3 H_2O: C, 64·47; H, 8·33; O, 27·09. Calc. for $C_{20}H_{30}O_6$: C, 65·55; H, 8·75; O, 26·20%. Found (after extensive drying): C, 65·45; H, 8·19; O, 26·41%).

Anhydrodihydroflexuosin B (XXIII). Prolonged treatment of XXI with ether at room temp. resulted in dehydration and conversion to XXIII, recrystallized from benzene–hexane, m.p. 120–122°, $[\alpha]_D^{24}$ +43·9° (*c*, 2·10), IR bands at 1775 (γ-lactone), 1725 and 1580 (ester and cyclopentenone) and 1180 cm^{-1} (ester), (nujol) 1775, 1745, 1715, 1575, 1180 and 1170 cm^{-1}, λ_{max} 227 mμ and 318 mμ (ε 19400 and 46), NMR signals at 7·67 dd (6,2, H_2), 6·07 dd (6,3, H_3), 5·45 br (H_6), 4·74 c (H_8), 1·5 d (7) and 1·21 d (7, C_{10}- and C_{11}-methyl), 1·04 (C_5-methyl), 0·88 ppm d (7, intensity 6 protons, terminal methyls of isovalerate). Found: C, 68·92; H, 7·99; O, 23·35. Calc. for $C_{20}H_{28}O_5$: C, 68·94; H, 8·10, O, 22·96%).

Anhydroflexuosin B (XXII). A solution of 0·111 g flexuosin B in 0·5 ml benzene containing 0·049 g *p*-toluenesulfonic acid monohydrate was refluxed 1 hr, cooled, diluted with ether, washed, dried and evaporated. The residue was recrystallized from acetone–hexane, m.p. 195–197°, $[\alpha]_D^{24}$ −134° (*c*, 0·91), IR bands at 1775 (γ-lactone), 1717 (cyclopentenone and unsaturated ester), 1650 (conjug. double bond), 1575 (double bond of cyclopentenone), 1174 and 1145 cm^{-1} (ester), NMR signals at 7·62 dd (6,2, H_2), 5·96 dd (6,3, H_3), 5·40 br (2 protons, H_6 and vinyl hydrogen superimposed), 4·70 d (7,2, H_8), 2·04 d (1) and 1·77 d (1, vinyl methyls), 1·42 d (7) and 1·15 d (7, C_{10}- and C_{11}-methyl), and 0·94 ppm (C_5-methyl). Repetition of this experiment with 4·4 g crude flexuosin B gave colored

material which was chromatographed over alumina (1·5 × 24 cm). Benzene eluted 2·3 g solid which after several recrystallizations from acetone–hexane furnished 1·76 g XXII, m.p. 194–198°. (Found: C, 69·51; H, 7·86; O, 23·03. Calc. for $C_{30}H_{36}O_5$: C, 69·34; H, 7·57; O, 23·09%).

Tetrahydroanhydroflexuosin B (XXIV). A solution of 0·197 g XXII in 17 ml ethanol was reduced with 0·072 g prereduced 5% Pd-C catalyst. Hydrogen uptake ceased after absorption of 2 mole-eqvts. (26·5 ml). The solution was filtered and evaporated; the residue, wt. 0·194 g crystallized on refrigeration and trituration with ether, m.p. 86–88°, $[\alpha]_D^{24}$ +41·1° (*c*, 1·07), positive Zimmermann test, IR bands (CCl_4) 1780 (γ-lactone), 1750 (cyclopentanone and ester), 1188 and 1770 cm^{-1}, λ_{max} 285 mμ, (ε 39). The same substance was obtained by catalytic hydrogenation of anhydrodihydroflexuosin B. (Found: C, 68·20; H, 8·45; O, 23·12. Calc. for $C_{30}H_{36}O_5$: C, 68·54; H, 8·63; O, 22·83%).

A sample of XXIV was synthesized by heating 0·26 g tetrahydrohelenalin, 0·15 g isovaleroyl chloride and 1 ml dry pyridine on the steam bath for 1 hr (protection from moisture). Pyridine was removed (red. press.), the residue decomposed with ice and water and the neutral fraction, wt. 0·2 g, chromatographed over alumina. The first 100 ml eluate furnished a gum which crystallized from ether–pet. ether, m.p. 83°, identical with XXIV by mixed m.p., IR spectrum and thin layer chromatography.

Hydrolysis of XXIV. A solution of 0·3 g XXIV in 21 ml 5% methanolic KOH was kept for 24 hr at room temp. (N_2 atm.), partially evaporated, diluted with water and extracted with chloroform. The aqueous layer was acidified with dil. HCl and extracted with ether. Evaporation of the organic layer furnished a gum which partially solidified. It was washed with a small amount of cold ether and recrystallized from acetone–hexane, yield 0·1 g m.p. 169–170°. The IR spectrum of this material was identical in all respects with the IR spectrum of dihydromexicanin C (XXV). A mixed m.p. with a sample of XXV, m.p. 172–173° was undepressed and the 2 samples were indistinguishable on thin-layer chromatography.

The odor of isovaleric acid was distinctly noticeable in the ether washings. They were evaporated, esterified with diazomethane and the ester compared with an authentic sample of methyl isovalerate by vapor phase chromatography. The 2 samples had the same retention time and when mixed gave only one peak.

Reaction of dihydromexicanin C with isovaleroyl chloride in the manner described for tetrahydrohelenalin gave a gum which could not be crystallized, but gave a single spot on thin-layer chromatography. It differed from XXIV in chromatographic behaviour and IR spectrum.

Note added in proof—That the actual structures are mirror images of the formulae given in this paper has recently been confirmed by the Bijroet technique (private communication from Dr. D. Rogers, Imperial College of Science and Technology, whom we thank for making this information available to us prior to publication).