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GAFRININ, A SESQUITERPENOID LACTONE FROM *GEIGERIA AFRICANA* GRIES—I

REVISED STRUCTURE

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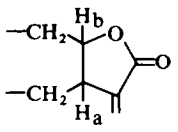
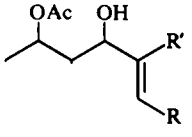
Abstract—Structure IIa, based on chemical and spectroscopic evidence, is proposed for the sesquiterpenoid lactone gafrinin, isolated from *Geigeria africana* Gries. Conversion to the known sesquiterpenoid lactone xanthumin established its configuration.

THE sesquiterpenoid lactone gafrinin was previously isolated from *Geigeria africana* Gries and the germacrane type structure I proposed for this compound.[1] As subsequent NMR spectra and chemistry contradicted this structure, the following evidence is put forward in support of IIa as the structure for gafrinin.

The previous molecular formula, $C_{17}H_{24}O_5$, for gafrinin, m.p. 110–111°, $[\alpha]_D$ −16°, was confirmed by mass spectrometry. The five oxygen atoms are readily characterized by spectroscopic methods. They are present as an OH group (IR absorption at 3509 cm^{-1}), an acetoxy substituent (IR max at 1701 and 1250 cm^{-1}, NMR 3-proton singlet at $\tau = 7{\cdot}99$), and a γ-lactone ring [IR band at 1745 cm^{-1}, UV max at 204 mμ (ε 14,100)].



A



B

The CO group of the γ-lactone is in conjugation with an exocyclic methylene group as shown in the partial structure A. The α,β′-unsaturated lactone is frequently found in sesquiterpenoid lactones[1–8] and is unambiguously identified by the IR and UV data given above and by its NMR spectrum which shows two vinyl proton resonances at $\tau = 3{\cdot}76$ (doublet, $J = 3{\cdot}3$ c/s) and $\tau = 4{\cdot}48$ (doublet, $J = 2{\cdot}7$ c/s) (NMR data are summarized in Table 1). Structure A is also supported by the following chemical evidence:

Hydrogenation of gafrinin with Pd–$CaCO_3$ gave a dihydro derivative III in which

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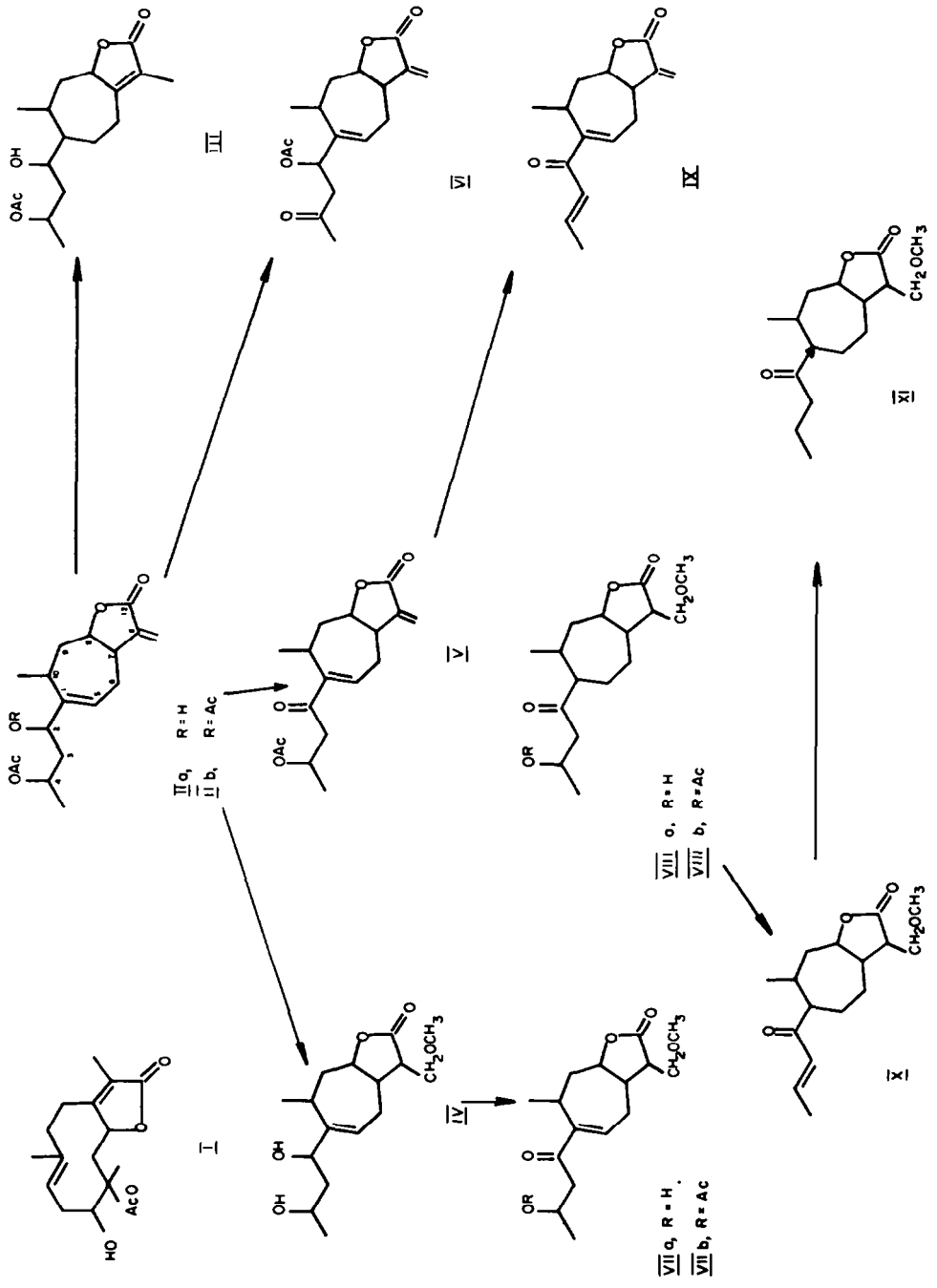

I
II a, R = H
II b, R = Ac
III
IV
V
VI
VII a, R = H
VII b, R = Ac
VIII a, R = H
VIII b, R = Ac
IX
X
XI
AcO
HO
OAc
OH
OR
CH2OCH3

the exocyclic methylene double bond had moved into the ring, indicated by the UV absorption max at 219 mμ (ε 6600)[9] and by the appearance of a new C-methyl group on a double bond at $\tau = 8{\cdot}2$ in the NMR spectrum, while the doublets at $\tau = 3{\cdot}76$ and 4·48 had disappeared. Gafrinin furthermore gave a crystalline pyrazoline[10] with diazomethane and ozonolysis of gafrinin gave rise to formaldehyde. Treatment of gafrinin with sodium methoxide in dry methanol led to deacetylation and a Michael addition of methanol over the exocyclic methylene group as described by Herz *et al.*[11] (compound IV). The doublets of the exocyclic methylene protons had again disappeared in the NMR spectrum and new resonances for the O-methyl (singlet, $\tau = 6{\cdot}69$) and O-methylene groups (doublet, $\tau \sim 6{\cdot}35$, S = 4·0 c/s) were observed.

From the multiplicity of the NMR signals of the protons H_a and H_b (Table 1, H-7 and H-8, respectively) follows that both protons are next to methylene groups thus establishing the partial structure A.

The presence of a trisubstituted double bond was clearly indicated by an absorption band at 813 cm^{-1} in the IR spectrum. This was confirmed by a further vinyl proton signal at $\tau = 4{\cdot}27$ in the NMR spectrum which showed two splittings of 6·4 and 8·4 c/s reflecting the neighbourhood of a methylene group.

It can now be concluded from the sum formula, functional groups and number of double bonds that gafrinin has a monocyclic structure. A 4-carbon (butyl) side-chain was proved by ozonolysis of gafrinin followed by periodate oxidation which gave β-acetoxy n-butyric acid, identified as its methyl ester by comparison (IR and gas chromatography) with an authentic sample.* The presence of a $CH_3CH(OAc)$-grouping accounts for one of the two secondary Me signals in the NMR spectrum ($\tau = 8{\cdot}73$ and 8·82).

The Michael adduct IV gave an isopropylidene derivative with acetone and sulphuric acid, thus showing the 1,3-relationship between the two oxygen functions on the side-chain of gafrinin, but, contrary to the findings of De Villiers,[1] no periodate was consumed when the compound was treated with sodium metaperiodate in aqueous ethanol.

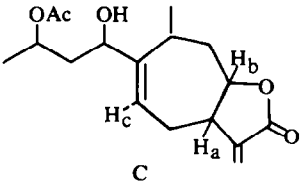
The allylic relationship between the OH group and the trisubstituted double bond became evident when, upon oxidation of gafrinin with manganese dioxide in chloroform, an α,β-unsaturated keto-acetate V, λ_{max} 231 mμ (ε 11,800) and ν_{max} 1660 cm^{-1} was obtained. Oxidation of the Michael adduct IV under the same conditions gave an α,β-unsaturated ketol VIIa, λ_{max} 235 mμ (ε 9600) and ν_{max} 1660 cm^{-1}. Further support is supplied by the NMR data as the τ-value of the olefinic proton H-5 in compounds V and VII is approximately 1 ppm lower (~3·2) than for other compounds (II, IV, VI, ~4·2), a deshielding effect which is explained by the mesomeric polarization of the enone system ($^{\oplus}C{=}C{-}C{=}O^{\ominus}$).

The keto-acetate V underwent β-elimination of acetic acid upon treatment with sodium acetate in boiling ethanol to give the cross-conjugated dienone IX, λ_{max} 241 mμ (ε 14,000) and ν_{max} 1660 and 1620 cm^{-1}, thus supporting the 1,3-relationship of the hydroxy and acetoxy functions.

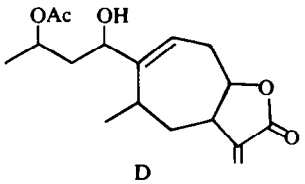
A second fragment B where R is attached to it via a methylene group can now be formulated. We are faced with the problem of deriving the structure of gafrinin from

* A mass spectrum of the α,β-unsaturated ketone X showed a base peak at *m/e* 69 due to the fragment, $CH_3{-}CH{=}CH{-}C{\equiv}O^+$. The corresponding fragment in the saturated ketone XI appeared at *m/e* 71, supplying additional proof for the Bu side-chain.

the fragments A and B with incorporation of a further secondary Me group ($CH—CH_3$). Only the two structures C and D are in agreement with all the spectroscopic and chemical evidence reported so far.



C



D

It has been shown by NMR decoupling experiments (see following paper) that the protons H_a and H_c are next to the same methylene group, thus proving structure C to be correct.

When the unsaturated ketol methyl ether VIIa was hydrogenated over Pd–$CaCO_3$, a saturated β-ketol methyl ether VIIIa was obtained, λ_{max} 283 mμ (ε 37) and ν_{max} 1700 cm^{-1}. Treatment of this compound with dilute alkali resulted in the formation of acetaldehyde through retro aldolization of the β-ketol. The acetaldehyde was identified by means of its 2,4-dinitrophenylhydrazone. A β-elimination of the OH group could also be indicated by the appearance of an absorption band at 227 mμ (ε 1440) when the UV spectrum of VIIIa was recorded in 0·1N ethanolic potassium hydroxide solution.

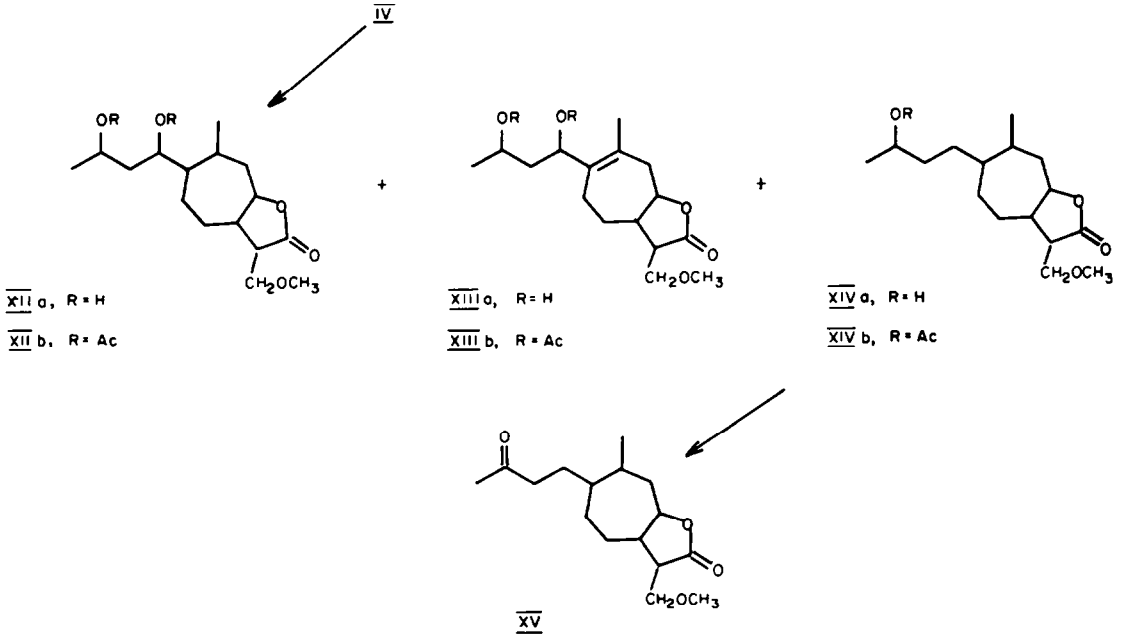
Acetylation of VIIIa with acetic anhydride-pyridine at room temperature gave an oily acetate VIIIb. Treatment of this compound with anhydrous sodium acetate and acetic anhydride at 90°, or adsorption of the acetate on neutral alumina for two days, gave a crystalline α,β-unsaturated ketone X, λ_{max} 227 and 300 mμ (ε 12,400 and 73, respectively). ν_{max} 1770, 1697, 1668 and 1635 cm^{-1}. The NMR resonances of the two olefinic protons of the α,β-unsaturated ketone X appeared as an ABX_3-system due to further splitting by the adjacent Me group: $\tau_A = 3{\cdot}85$, $\tau_B = 3{\cdot}09$, $J_{AB} = 15{\cdot}7$ c/s, $J_{AX} = 6{\cdot}7$ c/s and $J_{BX} = 1{\cdot}5$ c/s.

The α,β-unsaturated ketone X absorbed one mole of hydrogen upon hydrogenation over Pd–C to give the saturated ketone methyl ether XI with a max absorption at 278 mμ (ε 70) in the UV and 1710 cm^{-1} in the IR.

Hydrogenation of the Michael adduct IV over Pd–$CaCO_3$ gave a mixture of compounds from which three crystalline and one oily compound could be isolated. The normal hydrogenation product XIIa appeared as two crystalline isomers with molecular formula, $C_{16}H_{28}O_5$. One isomer had a m.p. 129–130°, $[\alpha]_D$ +33° compared to a m.p. 105·5–106·5°, $[\alpha]_D$ +58° for the other. The latter isomer also showed an IR absorption band at 985 cm^{-1} which was lacking in the former. Both isomers were acetylated to give the isomeric diacetates XIIb.

The third crystalline component obtained from the hydrogenation mixture also possessed two OH groups and gave a diacetate on acetylation. The NMR spectrum showed a methyl 3-proton singlet at $\tau = 8{\cdot}26$ in addition to one secondary Me doublet at $\tau = 8{\cdot}80$. The former signal suggested a Me group on a fully substituted double bond. Structure XIIIa is suggested for this compound.

The oily hydrogenation product XIVa only possessed one OH group which could be readily acetylated to a monoacetate XIVb. Oxidation of XIVa with chromic acid



in acetone[12] gave an oily ketone XV which showed a ketone absorption at 276 mμ (ε 26) and 1717 cm^{-1} in the UV and IR spectrum, respectively. The NMR spectrum also showed a 3-proton methyl ketone signal at $\tau = 7{\cdot}86$ and the compound gave a positive iodoform test. The allylic OH had thus been removed by hydrogenolysis.

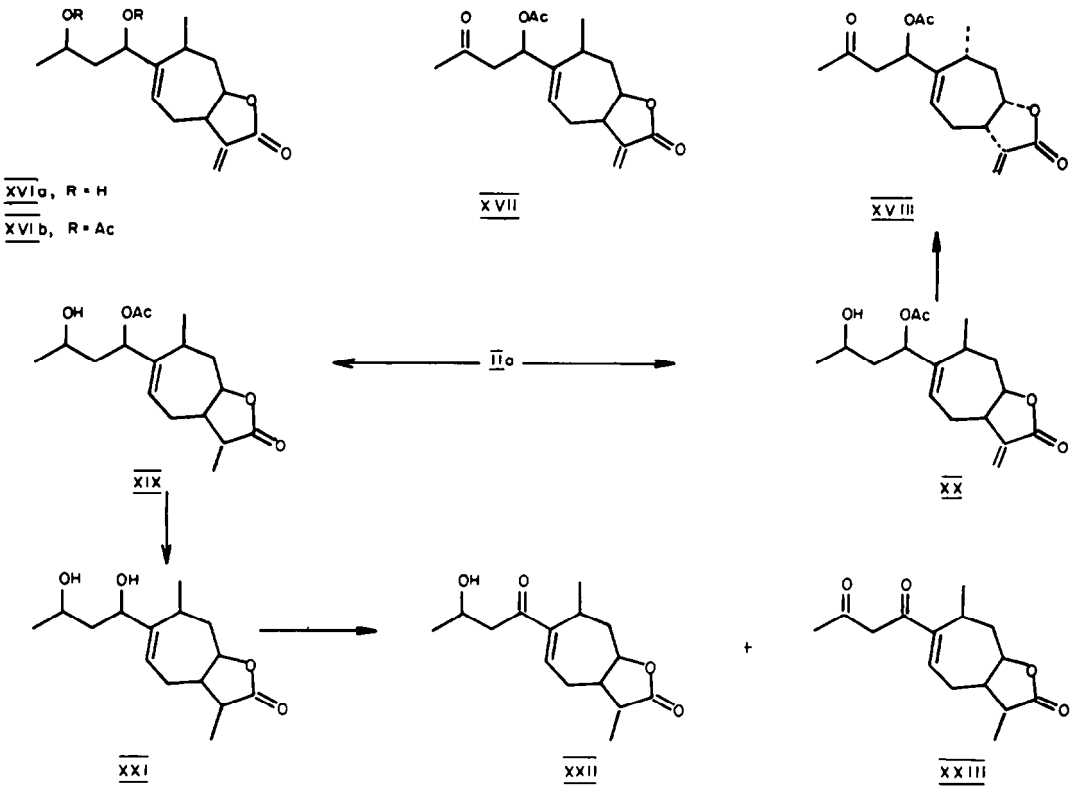
Attempts were finally made to correlate gafrinin with one of the sesquiterpenoids known to have an oxygenated butyl side-chain such as ivalbin XVIa,[13] xanthinin XVII[14] and xanthumin XVIII.[5]

The IR spectrum of gafrinin acetate IIb, m.p. 48–50°, $[\alpha]_D$ +19° differed considerably in the "fingerprint" region from that of ivalbin diacetate* XVIb, m.p. 111–112°, $[\alpha]_D$ −53·1°.

Oxidation of gafrinin IIa with manganese dioxide in chloroform gave, apart from the crystalline α,β-unsaturated keto-acetate V another crystalline compound VI, m.p. 98–99°, in small yield (ca. 7%). Its UV spectrum showed no α,β-unsaturated ketone absorption (no band between 230 and 240 mμ) whereas the IR spectrum (in CS_2) showed CO absorption at 1770 (α,β-unsaturated γ-lactone), 1745 (acetate) and 1735 cm^{-1} (side-chain ketone). The NMR spectrum showed a methyl ketone signal (singlet) at $\tau = 7{\cdot}81$ while the secondary Me signal at $\tau = 8{\cdot}73$ in gafrinin had disappeared. The signal due to an acetate at $\tau = 7{\cdot}99$ was still present and the multiplet at $\tau = 5{\cdot}05$ in gafrinin had now become a quartet at $\tau = 4{\cdot}59$ (S = 3·2 and 8·4 c/s). This compound VI was found to be identical (m.p., mixed m.p., IR, NMR and ORD) with an authentic sample of xanthumin.** Xanthumin[14] has been shown to be isomeric with xanthinin.

* The authors are very grateful to Professor W. Herz for a sample of this compound.

** The authors wish to thank Prof. H. Minato of the Shionogi Research Laboratory, Fukushima-Ku, Osaka, Japan, for a sample and NMR spectrum of xanthumin and an IR spectrum of the sodium borohydride reduction product of his xanthumin.



The conversion of gafrinin to xanthumin could also be effected by absorbing gafrinin in chloroform on alumina overnight whereupon a mixture consisting of unchanged gafrinin and compound XX was obtained. In the latter compound the OH and Ac functions of gafrinin had interchanged as could be determined from NMR evidence. Oxidation of this mixture with chromic acid in acetone gave a mixture of V and xanthumin XVIII, the yield of the latter being ca. 12% based on starting material.

It was also found that a reduction of gafrinin with sodium borohydride[13] gave an oily product XIX in which not only the exocyclic methylene group had been reduced (NMR: three Me doublets at τ = 8·81–8·84), but also the OH and Ac groups had been interchanged (NMR: 4-line pattern at τ = 4·92 (CH(OAc)—CH_2) and sextet at τ = 6·26 (CH_3—CH(OH)—CH_2)).

The IR spectrum of XIX was identical with the IR spectrum of the sodium borohydride reduction product of xanthumin. The compounds XXI–XXIII were also prepared for comparison with similar compounds from xanthumin.

The enedione XXIII was obtained as a minor product in the oxidation of the diol XXI with manganese dioxide in benzene. The enolizable β-diketone gave a strong colour reaction with ferric chloride, showed a strong UV absorption (in 0·1N ethanolic alkali) at 308 mμ (ε 17,400) and a broad CO absorption band at 1605 with weaker bands at 1661 and 1718 cm^{-1} in the IR.

The configuration of xanthumin[5] at C_7, C_8 and C_{10} was shown to be α so that the configuration of gafrinin at these centres must now also be α.

TABLE 1. NMR DATA ON GAFRININ AND ITS DERIVATIVES*

Protons	Compound IIa	IIb	IV	V
H-2	5·94 (q) S: 5·7, 7·9	4·92 (q) S: 6·0, 7·8	5·83 (q) S: 3·4, 9·0	—
H-3	—	—	—	—
H-4	5·05 (c) $J_{CH\ CH_3}$ = 6·2 S: 6·1, 7·5	5·13 (c) J_{CH-CH_3} = 6·2 S: ~6·5, 6·5	~6·0	4·77 (h) S: ~6·4
H-5	4·27 (q) S: 6·4, 8·4	4·20 (q) S: 6·4, 8·0	4·31 (t) S: ~7·4	3·26 (q) S: 6·5, 8·1
H-7	6·66 (c) S: 2·7, 3·3, 6·0, 8·6, 11·0	~6·7 (c)	—	—
H-8	5·37 (c) S: 3·0, 8·6, 11·6	5·39 (c) S: 4·1, 8·2, 10·3	5·52 (c) S: 3·2, 8·4, 11·5	5·41 (c) S: 4·1, 8·4, 11·2
H-9	—	—	—	—
H-13	3·76 (d) J = 3·3	3·78 (d) J = 3·3	~6·35 (d) S: 4·0	3·77 (d) J = 3·0
	4·48 (d) J = 2·7	4·50 (d) J = 2·7	6·69 (s, OMe)	4·46 (d) J = 2·8
C-4 Me	8·73 (d) J = 6·2	8·74 (d) J = 6·2	8·83 (d) J ~ 6·0	8·74 (d) J = 6·3
C-10 Me	8·82 (d) J = 6·8	8·86 (d) J = 6·8	8·83 (d) J ~ 6·0	8·97 (d) J = 6·8
OAc	7·99 (s)	7·98 (s); 7·99 (s)	—	8·02 (s)

TABLE 1—*continued*

Protons	Compound VI		Compound VIIa		Compound VIIb		Compound VIIIa	
H-2	4·62 (X)	J_{AB} = 16·5 J_{AX} = 8·5	—		—		—	
H-3	7·16 (A) 7·34 (B)	J_{BX} = 4·9	7·27 (A) 7·28 (B)	J_{AB} = 15·5 S_{AX} = 6·8 S_{BX} = 4·5	7·17 (A) 7·53 (B)	J_{AB} = 15·9 J_{AX} = 6·8 J_{BX} = 5·9	7·37 (A) 7·52 (B)	J_{AB} = 17·8 J_{AX} = 8·3 J_{BX} = 3·1
H-4		—	5·79 (X, c)	J_{CH-CH_3} = 6·2	4·78 (X, c)	J_{CH-CH_3} = 6·2	5·78 (X, c)	J_{CH-CH_3} = 6·2
H-5	4·20 (q)	S: 6·1, 8·5	3·19 (q)	S: 6·1, 8·6	3·25 (q)	S: 6·2, 8·7		—
H-7	~6·8 (c)		~7·5		~7·6			—
H-8	5·39 (c)	S: 4·5, 8·1, 10·5	5·53 (c)	S: 3·3, 8·3, 11·5	5·56 (c)	S: 3·4, 8·1, 11·4	5·34 (c)	S: 4·4, 9·0, 9·5
H-9		—	7·92 (A) 8·13 (B)	ABXY; J_{AB} = 13·8 $S_{A,8}$ = 3·3; $S_{A,10}$ = 7·5 $S_{B,8}$ = 11·5; $S_{B,10}$ ~ 0	7·91 (A) 8·15 (B)	ABXY; J_{AB} = 13·8 $S_{A,8}$ = 3·4; $S_{A,10}$ = 7·4 $S_{B,8}$ = 11·5; $S_{B,10}$ ~ 0		—
H-13	3·81 (d) 4·51 (d)	J = 3·2 J = 2·8	6·32 (d) 6·67 (s, OMe)	S: 4·5	6·38 (d) 6·68 (s, OMe)	S: 4·5	6·37 (ABX), 6·67 (s, OMe)	J_{AB} = 9·8 S_{AX} = 3·8 S_{BX} = 4·7
C-4 Me	7·84 (s)		8·82 (d)	J = 6·2	8·75 (d)	J = 6·2	8·82 (d)	J = 6·2
C-10 Me	8·86 (d)	J = 6·7	8·93 (d)	J = 6·8	8·95 (d)	J = 6·7	9·08 (d)	S: 5·8
OAc	8·01 (s)			—	8·13 (s)			—

TABLE 1—*continued*

Protons	Compound VIIIb		X		XI		XIX	
H-2	—		—		—		4·92 (q)	S: 5·6, 7·8
H-3	7·08 (A)	J_{AB} = 17·2	3·85 (B)	ABX_3	—			—
	7·50 (B)	J_{AX} = 7·2		J_{AB} = 15·7				
		J_{BX} = 5·6		J_{AX} = 6·7				
H-4	4·75 (X, c)	J_{CH-CH_3} = 6·4	3·09 (A)	J_{BX} = 1·5	—		6·26 (h)	S: ~6·7
H-5	—		—		—		4·28 (q)	S: 4·2, 8·6
H-7	—		7·23 (c)		7·23 (c)			—
H-8	5·36 (c)		5·33 (c)		5·34 (c)		5·46 (c)	
H-9	—		—		—			—
H-13	6·38 (ABX)	J_{AB} ~ 9·5	6·36 (ABX),	J_{AB} = 9·6	6·36 (ABX),	J_{AB} = 9·6	8·84†(d)	J = 6·8
				S_{AX} = 3·6		S_{AX} = 3·6		
	6·68 (s, OMe)			S_{BX} = 4·8		S_{BX} = 4·8		
			6·68 (s, OMe)		6·66 (s, OMe)			
C-4 Me	8·73 (d)	J = 6·4	8·10 (d, d)	J = 6·7, 1·5	9·11 (t)	J = 7·0	8·81 (d)	J = 6·3
C-10 Me	9·08 (d)	S: 5·6	9·10 (d)	S: 5·8	9·11 (d)	S: 5·7	8·83†(d)	J = 7·0
OAc	8·03 (s)		—		—		8·01 (s)	

Abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; h = hextet; c = complex; S = splitting.

* Chemical shifts are measured on the τ-scale; coupling constants and splittings are given in c/s.

† Assignments may be interchanged.

EXPERIMENTAL

M.ps are uncorrected. Unless otherwise stated, $[\alpha]_D$ and UV absorption spectra refer to EtOH, IR spectra to $CHCl_3$ and NMR spectra to $CDCl_3$ solns. IR absorption spectra were recorded on Perkin–Elmer models 21 and 237 spectrometers, UV absorption spectra on a Unicam model S.P. 800 spectrometer and NMR spectra on Varian A-60 and HA-100 spectrometers. Chemical shifts were measured on the τ-scale relative to TMS as internal standard (τ = 10·0); τ-values are estimated to be accurate to ±0·01 ppm, coupling constants to ± 0·2 c/s. Mass spectra were recorded on a MS-9 spectrometer.

The β-acetoxy methyl butyrate was identified on a Beckman GC-2 gas chromatograph using a Beckman 74346 G.1. partition chromatography column (silicone).

TLC was carried out on silica gel plates using $CHCl_3$–MeOH (19:1) as solvent system. The spots were developed with the vanillin-phosphoric acid reagent or with 0·5% $KMnO_4$ in saturated copper acetate.

Geigeria africana was obtained from the Rietondale Experimental Farm, Pretoria, through the courtesy of Dr. T. Terblanche of Onderstepoort.

Extraction and isolation of gafrinin. The method of isolation of IIa from the plant was essentially the same as that employed by De Villiers.[1] Ground, air-dried *G. africana* (32 kg) gave, after extraction with 96% EtOH and subsequent purification with basic lead acetate and chromatography on cellulose powder impregnated with formamide (40% in acetone), IIa (8·9 g) as fine, colourless needles from acetone ether, m.p. 110–11°, $[\alpha]_D$ −16° (*c* 1·0), λ_{max} 205 mμ (ε 14,100), ν_{max} 3509 (OH), 1745 (α,β-unsaturated γ-lactone), 1701 and 1250 (acetate), 1645 and 813 cm^{-1} (trisubst. C=C). [Found: C, 66·4; H, 7·9; *M* (mass spect.) 308. Calc. for $C_{17}H_{24}O_5$: C, 66·2; H, 7·9%; *M*, 308.]

Acetylation of IIa with Ac_2O–pyridine at room temp overnight gave, after chromatography on formamide-impregnated cellulose powder, IIb as an oil which crystallized slowly. The crystals were washed with cold ether: m.p. 48–50°, $[\alpha]_D$ +19° (*c* 0·73), $\nu_{max}^{CS_2}$ 1775 (α,β-unsaturated γ-lactone), 1742 and 1240 cm^{-1} (acetate). (Found: C, 65·0; H, 7·2. Calc. for $C_{19}H_{26}O_6$: C, 65·1; H, 7·4%.)

Hydrogenation of gafrinin IIa. Gafrinin (1 g) absorbed 0·9 mole H_2 upon hydrogenation in EtOH over Pd–$CaCO_3$ to give oily III (295 mg) after purification by chromatography on formamide-impregnated cellulose in hexane-benzene (1:1), λ_{max} 219 mμ (ε 6600), ν_{max} 1755 cm^{-1} (α,β-unsaturated γ-lactone). (Found: C, 65·6; H, 8·7. Calc. for $C_{17}H_{26}O_5$: C, 65·8; H, 8·4%.)

Pyrazoline derivative[4] *of gafrinin.* A suspension of IIa (100 mg) in abs ether (100 ml) was treated with diazomethane prepared from nitrosomethyl urea (350 mg). Another portion of diazomethane was added after one day and, after three more days in the refrigerator, the solvent was removed and the residue crystallized from $CHCl_3$–ether to give colourless *crystals* (63 mg), m.p. 97–98°. (Found: C, 61·8; H, 7·5; N, 8·0. $C_{18}H_{26}O_5N_2$ requires: C, 61·7; H, 7·5; N, 8·0%.)

Ozonolysis of gafrinin

(a) Gafrinin (100 mg) in AcOH (25 ml) was treated with a stream of O_2 containing 3 mg of O_3/min for 90 min. The mixture was steam-distilled into aqueous dimedone. Upon concentration, a ppt was formed (24 mg) which had m.p. 191° alone or mixed with the dimedone derivative of formaldehyde.

(b) Gafrinin (944 mg; *ca.* 3 mmoles) was dissolved in EtOAc (50 ml) and treated with O_3 for 1¾ hr at 0°. The EtOAc was removed *in vacuo* and the crude, yellow ozonide (ca. 1 g) refluxed under N_2 with hot water (20 ml) for 15 min. MeOH (15 ml) was added, followed by sodium metaperiodate (1·24 g) in water (10 ml). The soln was kept overnight, diluted with water and SO_2 passed through for 15 min. This was followed by a continuous extraction with ether which was subsequently treated with a 5% Na_2CO_3 aq. Acidification of the carbonate soln followed by a continuous extraction with ether and evaporation of the latter gave an oily acid fraction (26 mg) with a butyric acid odour, ν_{max} 3500 (broad: OH), 1730 (carboxyl), 1730 and 1260 cm^{-1} (acetate).

Esterification with diazomethane gave the methyl ester of β-acetoxy butyric acid, ν_{max} 1735 and 1725 (inflexion), (methyl ester and acetyl), 1250 cm^{-1} (acetate). Gas chromatography (temp, 220°; flow rate 480 ml/min) showed only one peak at a retention time of 31·5 min.

This product was identical in IR and gas chromatography with an authentic sample prepared from the Na salt of β-acetoxy butyric acid (BDH) by treatment with acetyl chloride and subsequently with diazomethane in ether.

Preparation of the Michael adduct IV *from gafrinin.* Treatment of IIa with NaOMe in dry MeOH according to De Villiers[1] gave IV, m.p. 93–94° (from acetone-ether), $[\alpha]_D$ −40° (*c* 1·0), λ_{max} 205 mμ (ε 3000), ν_{max} 3480 (hydroxyl) and 1770 cm^{-1} (saturated γ-lactone). (Found: C, 64·4; H, 8·6. Calc. for $C_{16}H_{26}O_5$: C, 64·4; H, 8·7%.)

Isopropylidene derivative of IV. To a soln of IV (201 mg) in AR acetone (50 ml) was added 20 drops of an 8N H_2SO_4 mixture (2·3 ml H_2SO_4 in 10 ml water) and the mixture then left at room temp for 4½ hr. It was then diluted with water and extracted with $CHCl_3$ which was subsequently dried over Na_2SO_4 and removed *in vacuo*. The residual oil (197 mg) was chromatographed on silica gel (20 g) in $CHCl_3$–MeOH (99·5:0·5) and the fractions with R_f 0·8 combined. The solvent was removed *in vacuo* and the residue distilled to give a colourless oil, b.p. 120–130°/0·5 mm, $[\alpha]_D$ −15·4° (*c* 0·67), $\nu_{max}^{CS_2}$ 1790 (saturated γ-lactone) and 1380 cm^{-1} (*gem*-dimethyl). (Found: C, 67·4; H, 8·9. Calc. for $C_{19}H_{30}O_5$: C, 67·4; H, 8·9%.)

Oxidation of IV *with* MnO_2. The adduct IV (394 mg) was shaken with a suspension of MnO_2 (4 g; prepared according to Attenburrow[15]) in $CHCl_3$ (40 ml) for 5 days at 19°. The MnO_2 was filtered off on "hyflo supercel" and washed with hot $CHCl_3$. The filtrate and washing were combined and evaporated to give a colourless oil (360 mg) after purification with animal charcoal. This oil was chromatographed on formamide-impregnated cellulose (40 g). Hexane-benzene (1:1) eluted fractions which showed only one spot (R_f 0·55) on chromatoplates. The fractions were combined and evaporated to give an *oil* (VIIa; 180 mg), λ_{max} 235 mμ (ε 9600) and 310 mμ (ε 49), ν_{max} 3600 (OH), 1770 (saturated γ-lactone) and 1660 cm^{-1} (α,β-unsaturated ketone).

Acetylation of VIIa with Ac_2O and pyridine overnight at room temp gave crystalline VIIb, m.p. 96–98°, $[\alpha]_D$ −73° (*c* 0·58), ν_{max} 1770 (saturated γ-lactone), 1660 (α,β-unsaturated ketone). 1760 and 1250 cm^{-1} (acetate). (Found: C, 63·8; H, 7·8. $C_{18}H_{26}O_6$ requires: C, 63·9; H, 7·7%.)

Hydrogenation of the α,β-unsaturated ketone VIIa. The oily VIIa (592 mg) absorbed 1·05 moles H_2 upon hydrogenation over 5% Pd-C (500 mg) in 96% EtOH. The product (550 mg) was chromatographed on formamide-impregnated cellulose.

Hexane-benzene (1:1) eluted an *oil* (VIIIa; 415 mg; R_f 0·6) which crystallized from ether, m.p. 58·5–59°, $[\alpha]_D$ +39° (*c* 0·55), λ_{max} 283 mμ (ε 37), $\lambda_{max}^{0·1N-KOH}$ 227 mμ (ε 1440); ν_{max} 3340 (OH), 1760 (γ-lactone) and 1700 cm^{-1} (ketone). (Found: C, 64·1; H, 8·8. $C_{16}H_{26}O_5$ requires: C, 64·4; H, 8·8%.)

Acetylation of VIIIa with Ac_2O–pyridine at room temp overnight, followed by chromatography over formamide-impregnated cellulose and subsequent distillation of the acetate in high vacuum, gave an *oil* VIIIb, R_f 0·7, $[\alpha]_D$ +16·4° (*c* 0·61), λ_{max} 284 mμ (ε 31), $\nu_{max}^{CS_2}$ 1787 (γ-lactone), 1752 and 1243 (acetate) and 1724 cm^{-1} (ketone). (Found: C, 63·3; H, 8·4. $C_{18}H_{26}O_6$ requires: C, 63·5; H, 8·3%.)

Retro aldolization of the β-ketol VIIa. Compound VIIa (100 mg) was covered with water (5 ml) and 3N NaOH (10 ml) added dropwise to it at room temp while a steady stream of N_2 was bubbled through and into a saturated 2,4-DNP solution in 4N HCl. The temp was afterwards raised to 50°. Orange crystals appeared in the 2,4-DNP soln and were filtered off and crystallized from dil EtOH to give acetaldehyde-2,4-DNP (7 mg), m.p. 159–161° which was identical with an authentic sample of acetaldehyde-2,4-DNP in m.p., mixed m.p. (158°) and IR.

β-Acetoxy elimination in VIIIb. Compound VIIIb (778 mg) was heated with freshly-fused NaOAc (900 mg) in Ac_2O (40 ml) at 90° for 1½ hr. The anhydride was decomposed in ice-water and the latter extracted with $CHCl_3$. The $CHCl_3$ phase was washed with water, dried over Na_2SO_4 and evaporated to dryness, leaving a yellow, crude oil (730 mg). Chromatography of this oil on formamide-impregnated cellulose (80 g) in hexane-benzene (19:1) gave a purified oily product X which crystallized as *needles* from ether–hexane, m.p. 54·5–56°, $[\alpha]_D$ +63·5° (*c* 0·6), λ_{max} 227 mμ (ε 12,400) and 300 mμ (ε 73), ν_{max} 1770 (γ-lactone), 1697, 1668 (*transoid* double bond) and 1635 cm^{-1} (*cisoid* double bond).[13] (Found: C, 68·5; H, 8·5. $C_{16}H_{24}O_4$ requires: C, 68·5; H, 8·6%.)

Hydrogenation of the α,β-unsaturated ketone X. Compound X (90 mg) absorbed 1·04 moles H_2 upon hydrogenation over 5% Pd–C (118 mg) in 96% EtOH. It was worked up in the usual manner and the oily product was decolorized with animal charcoal and then crystallized from ether–hexane to give waxy *crystals* of XI, m.p. 44–46°, $[\alpha]_D$ +37·5° (*c* 0·58), λ_{max} 278 mμ (ε 70), $\nu_{max}^{CS_2}$ 1775 (γ-lactone) and 1710 cm^{-1} (ketone). (Found: C, 67·8; H, 9·3. $C_{16}H_{26}O_4$ requires: C, 68·1; H, 9·3%.)

Hydrogenation of the Michael adduct IV. Compound IV (756 mg) was hydrogenated over 5% Pd-$CaCO_3$ (740 mg) in 96% EtOH and 1·06 moles H_2 was absorbed. The catalyst was filtered off, the EtOH removed *in vacuo* and the residue (650 mg) chromatographed on formamide-impregnated cellulose.

Hexane–benzene (1:1) eluted an *oil* (XIVa; 155 mg; R_f 0·7), b.p. 120°/10^{-4} mm, $[\alpha]_D$ +50·6° (*c* 0·7), ν_{max} 3480 (OH) and 1760 cm^{-1} (γ-lactone). (Found: C, 67·5; H, 10·1. $C_{16}H_{28}O_4$ requires: C, 67·6; H, 9·9%.)

Acetylation with cold Ac_2O–pyridine gave an oily *mono-acetate* XIVb, $[\alpha]_D$ + 28·8° (*c* 0·55), ν_{max} 1760 (γ-lactone), 1730 and 1240 cm^{-1} (acetate). (Found: C, 66·3; H, 9·2. $C_{18}H_{30}O_5$ requires: C, 66·2; H, 9·2%.)

Benzene eluted two *isomers* XIIa, m.p. 129–130° (from $CHCl_3$–ether), $[\alpha]_D$ +33° (*c* 0·5), ν_{max} 3550 (OH) and 1770 cm^{-1} (γ-lactone). (Found: C, 64·3; H, 9·5. $C_{16}H_{28}O_5$ requires: C, 64·0; H, 9·4%) and m.p.

105·5–106·5° (from $CHCl_3$–ether), $[\alpha]_D$ +58° (*c* 0·55), ν_{max} 3500 (OH), 1770 (γ-lactone) and 985 cm^{-1}. (Found: C, 64·1; H, 9·6. $C_{16}H_{28}O_5$ requires: C, 64·0; H, 9·4%.)

Benzene also eluted the crystalline *product* XIIIa, m.p. 142–143·5° (from $CHCl_3$–ether), R_f 0·20, $[\alpha]_D$ −20° (*c* 0·55), λ_{max} 203 mμ (ε 8400). (Found: C, 64·1; H, 8·9. $C_{16}H_{26}O_5$ requires: C, 64·4; H, 8·8%.)

Acetylation of this product with cold Ac_2O–pyridine gave a crystalline *diacetate* XIIIb, from $CHCl_3$–ether, m.p. 112–113°, $[\alpha]_D$ −37·6° (*c* 0·54), $\nu_{max}^{CS_2}$ 1760 (γ-lactone), 1730 and 1240 cm^{-1} (acetate). (Found: C, 62·6; H, 8·0. $C_{20}H_{30}O_7$ requires: C, 62·8; H, 7·9%.)

Oxidation of XIVa *to the methyl ketone* XV. Compound XIVa (490 mg) was dissolved in acetone (130 ml) and titrated with 8N chromic acid at 5° until 1·13 moles CrO_3 had been used. The mixture was left at 5° for ½ hr. whereupon MeOH and water were added and the product extracted with $CHCl_3$. The latter was washed with water, dried over Na_2SO_4 and evaporated to give an oil (490 mg). Chromatography on cellulose in hexane–benzene (3:1) gave an oil (372 mg) which was subsequently distilled at 120°/1·84 × 10^{-4} mm to give a pure methyl ketone *product* XV, R_f 0·6, $[\alpha]_D$ +48·4° (*c* 0·69), λ_{max} 276 mμ (ε 26), $\nu_{max}^{CS_2}$ 1775 (saturated γ-lactone) and 1717 cm^{-1} (ketone). (Found: C, 68·2; H, 9·2. $C_{16}H_{26}O_4$ requires: C, 68·1; H, 9·3%.)

Reduction of gafrinin with sodium borohydride. $NaBH_4$ (350 mg) was added to a soln of IIa (3 g) in MeOH (30 ml) and left at room temp for 2 hr. The excess borohydride was decomposed with 2N H_2SO_4 (6 ml) and the soln then extracted with $CHCl_3$ (7 × 10 ml). The $CHCl_3$ extract was washed with 2N Na_2CO_3, water and then dried over Na_2SO_4. After evaporation of the $CHCl_3$, an oily product (3 g) was obtained which was chromatographed on formamide-impregnated cellulose (300 g). Hexane–benzene (1:1) eluted a colourless oil (XIX; 2·67 g) which was purified by distillation, b.p. 140–145°/0·005 mm, $[\alpha]_D$ −39·2° (*c* 1·0), ν_{max} 3570 and 3490 (OH), 1764 (saturated γ-lactone), 1727 and 1255 cm^{-1} (acetate). [Found: C, 65·8; H, 8·4; *M* (mass spect.), 310. $C_{17}H_{26}O_5$ requires: C, 65·3; H, 8·2%; *M*, 310.]

De-acetylation of XIX. A soln of XIX (2·37 g) in 10% K_2CO_3–MeOH (34 ml) was refluxed for 1 hr and then evaporated. The residue was dissolved in water (80 ml), acidified with 2N acid and extracted with $CHCl_3$ (7 × 10 ml). The $CHCl_3$ was washed with water, dried and evaporated to give an oily residue (2·3 g) which was then chromatographed on formamide-impregnated cellulose. Benzene–hexane (9:1) eluted an *oil* (XXI; 1·2 g) which was further purified by distillation, b.p. 140–145°/0·003 mm, $[\alpha]_D$ −36·2° (*c* 1·0), ν_{max} 3580 and 3450 (OH's), and 1759 cm^{-1} (γ-lactone). [Found: C, 66·7; H, 8·9; *M* (mass spect.), 268. $C_{15}H_{24}O_4$ requires: C, 67·2; H, 9·0%; *M*, 268.]

Oxidation of XXI *with manganese dioxide.* A mixture of XXI (1 g) and MnO_2 (5 g) in benzene (50 ml) was shaken for 13 hr at room temp. Filtration and evaporation of the filtrate gave a viscous oil (750 mg) which was chromatographed on formamide-impregnated cellulose.

Benzene–hexane (1:3) eluted the *diketone* (XXIII; 183 mg) which was further purified by distillation, b.p. 100–105°/0·005 mm, $[\alpha]_D^{dioxan}$ −16·4° (*c* 0·7), λ_{max} 240 mμ (ε 5840) and 300 mμ (ε 8850), $\lambda_{max}^{0·1N-KOH}$ 308 mμ (ε 17,400), ν_{max} 1761 (γ-lactone), 1718, 1661 and 1605 cm^{-1} (enedione). [Found: *M* (mass spect.), 264. $C_{15}H_{20}O_4$ requires: *M*, 264.]

Benzene–hexene (3:1) eluted the β-*ketol* (XXII; 402 mg), b.p. 110–115°/0·005 mm, $[\alpha]_D^{dioxan}$ −45·7° (*c* 0·61), λ_{max} 234 mμ (ε 11,200), ν_{max} 3550 (OH), 1760 (γ-lactone) and 1660 cm^{-1} (α,β-unsaturated ketone). [Found: *M* (mass spect.), 266. $C_{15}H_{22}O_4$ requires: *M*, 266.]

Oxidation of gafrinin IIa *with manganese dioxide.* IIa (1·04 g) was oxidized with MnO_2 in $CHCl_3$ as described previously. The crude, oily product obtained (783 mg) was chromatographed on formamide-impregnated cellulose (100 g). Hexane–benzene (9:1) eluted an oil which crystallized from acetone as feathery *needles*, V, m.p. 73–75°, $[\alpha]_D$ −39·4° (*c* 0·45), λ_{max} 231 mμ (ε 11,800) and 205 mμ (ε 9770), $\nu_{max}^{CS_2}$ 1770 (α,β-unsaturated γ-lactone), 1660 (α,β-unsaturated ketone), 1730 and 1240 cm^{-1} (acetate). (Found: C, 66·4; H, 7·2. $C_{17}H_{22}O_5$ requires: C, 66·7; H, 7·2%.)

Hexane–benzene (3:1) eluted VI (56 mg), identical with xanthumin, which crystallized from $CHCl_3$–ether, m.p. 98–99°, λ_{max} 271 mμ (ε 50) and 204 mμ (ε 14,300), $\nu_{max}^{CS_2}$ 1770 (α,β-unsaturated γ-lactone), 1745 (acetate) and 1735 cm^{-1} (ketone). (Found: C, 66·3; H, 7·1. Calc. for $C_{17}H_{22}O_5$: C, 66·7; H, 7·2%.)

β-*Acetoxy elimination in compound* V. Compound V (100 mg) and freshly fused NaOAc (200 mg) was refluxed in abs EtOH (2·5 ml) for 2½ hr. Water was added and the soln extracted with $CHCl_3$ which was subsequently washed with water and dried over Na_2SO_4. Evaporation of the $CHCl_3$ gave an oily residue (80 mg) which was purified by distillation at 100°/0·005 mm to give the oily *dienone* IX, $[\alpha]_D$ +59° (*c*, 0·75), λ_{max} 205 mμ (ε 19,230), 241 mμ (ε 14,000) and 325 mμ (ε 98), ν_{max} 1755 (α,β-unsaturated γ-lactone), 1660 and 1620 cm^{-1} (dienone). [Found: *M* (mass spect.), 246. $C_{15}H_{18}O_3$ requires: *M*, 246.]

Conversion of gafrinin IIa *to xanthumin* XVIII *through absorption on alumina followed by oxidation with* CrO_3. Gafrinin (500 mg) was absorbed on activated alumina (Merck, Grade II; 50 g) in $CHCl_3$ for 2 days

at room temp. The mixture (500 mg) containing about 25% (based on NMR evidence) of the compound XX was washed from the column, dried *in vacuo* and oxidized with 8N chromic acid in acetone as previously described. The crude oxidation mixture (430 mg) was chromatographed on formamide-impregnated cellulose.

Hexane–benzene (9:1) eluted V (270 mg) and XVIII (60 mg). The latter was crystallized from ether–hexane and finally from $CHCl_3$–ether as fine needles (12 mg), m.p. 98–99°; ν_{max}^{KBr} 1755 (α,β-unsaturated γ-lactone), 1742 and 1240 (acetate), and 1721 (ketone). [Found: C, 67·0; H, 7·4; *M* (mass spect.), 306. Calc. for $C_{17}H_{22}O_5$: C, 66·7; H, 7·3%; *M*, 306.]

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