Tetrahedron. Vol 24, pp 3321 to 3328 Pergamon Press. 1968 Printed in Great Britain

THE ISOLATION AND STRUCTURE OF A NEW DITERPENE LACTONE FROM *TRICHOTHECIUM ROSEUM* LINK

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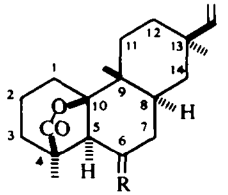
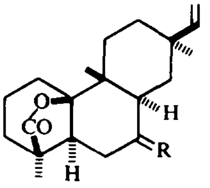
(*Received in the UK* 20 *October* 1967; *accepted for publication* 21 *November* 1967)

Abstract—A new diterpene isolated from *Trichothecium roseum* Link is formulated as 6β-hydroxyrosenonolactone on the basis of chemical and spectrochemical evidence. Circular dichroism data on lactones derived from the rosane skeleton are discussed.

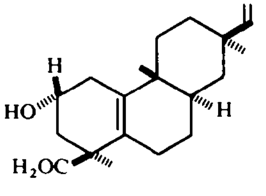
IN THE course of studies on the toxigenic activity of fungi, *Tricothecium roseum* Link was frequently isolated from stored foodstuffs. A strain of this species, isolated from groundnuts was found to cause acute toxicoses in ducklings. Maize-meal was used for the large-scale cultivation of this strain (designated G-432)[1] and the toxic principles were extracted quantitatively with chloroform-methanol. Fractionation of the extract was followed throughout by bio-assay on day-old ducklings. This revealed that the toxicity was due mainly to the accumulative effect of rosololactone (Ia),[2] ergosterol, and an unknown compound, $C_{20}H_{28}O_4$. Other diterpenes known to be elaborated by this fungus are rosenonolactone (IIa),[3] deoxorosenonolactone (IIb),[4] and isorosenolic acid (III).[5] The absolute stereochemistry of these compounds has recently been defined.[6] Compounds IIa and IIb were also produced by strain G-432, but did not contribute significantly to the total toxicity.



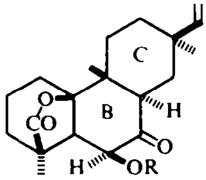
Ia : R = ▬OH, H
Ib : R = O
Ic : R = ▬OAc, H



IIa : R = O
IIb : R = H_2



III



IVa : R = H
IVb : R = Ac

The new metabolite,* $C_{20}H_{28}O_4$, is formulated as 6β-hidroxyrosenonolactone (IVa) on the basis of the following evidence. Its IR spectrum showed, in addition to hydroxyl [3578 (sh) and 3450 (br) cm^{-1}] and carbonyl (1718 cm^{-1}) absorption, a maximum at 1768 cm^{-1} characteristic of a γ-lactone and bands at 3070, 1638 and 918 cm^{-1} indicating the presence of a vinyl group. From PMR† patterns (a quartet around τ 4·16 and two superimposed quartets around τ 5·06) this vinyl group was attached to a fully substituted C atom. The PMR spectrum further showed three-proton singlets at τ 8·59, 8·80 and 9·09 ascribed to three quaternary-attached C—Me groups. These data suggested that the compound is a diterpene lactone of the type IIb. The position of the OH group was deduced from PMR data. A signal (one proton) at τ 7·02 is assigned to the OH proton. A quartet at τ 6·02 (J = 2·5 and 5·5 c/s) reduced to a doublet (J = 5·5 c/s) when the OH proton was exchanged with deuterium oxide. The proton on the C bearing the OH group is, therefore, coupled only to the OH proton and one other proton. Irradiation of this signal at τ 6·02 caused the collapse of a doublet (J = 5·5 c/s) at τ 7·71 to a singlet while the signal at τ 7·02 was sharpened considerably. Unless the stereochemistry of the system is such as to preclude observable spin coupling to other neighbouring protons, the spectroscopic data indicate that the OH proton must be located at position 6 and the keto group at position 7. On the basis of this assignment, a quartet at τ 7·39 is ascribed to the proton at position 8. Independent evidence for the presence of an α-ketol system in IVa was provided by its strong positive triphenyltetrazolium chloride reaction.

Compound IVa, like rosololactone (Ia) was not acetylated by prolonged treatment with acetic anhydride–pyridine at room temperature. This may be due to severe steric hindrance of the OH group in these compounds. However, quantitative acetylation of Ia and IVa was achieved by reaction with acetic anhydride using boron trifluoride as catalyst. The PMR spectrum of the acetate of IVa showed *inter alia* a three-proton singlet at τ 7·96 due to the acetoxyl group, a doublet (J = 5·5 c/s) at τ 4·62 due to C_6—H and a doublet (J = 5·5 c/s) at τ 7·44 due to C_5—H overlying the quartet of the proton at C-8. Clearly, acetylation took place without isomerization to give IVb.

Compound IVa was correlated with the known diterpene lactones of *T. roseum* by reaction of its acetate IVb with zinc in acetic acid.[8] The main product of this reaction was identified as rosenonolactone (IIa). This experiment also confirmed the presence of a 6-hydroxy-7-keto grouping and an A/B *trans* ring juncture in IVa. Since the proton on C-5 has an axial orientation, the coupling constant (5·5 c/s) between C_5—H and C_6—H is only compatible with an approximate axial-equatorial orientation[9] of these protons. The OH group at C-6 must therefore have a 6β (axial) configuration. The above correlation does not unambiguously establish the stereochemistry of the B/C ring juncture, since the possibility of isomerization[3] at C-8 cannot be precluded. On biogenetic grounds, IVa would be expected to have a *trans* B/C ring juncture. The coupling constants (J = 4·5 and 10·5 c/s) between C_8—H and C_{14}—H_2 indicate that C_8—H lies outside the *gem*-protons as required for a *trans* C/D ring juncture.[10] The absolute stereochemistry IVa can therefore be written for the new diterpene lactone.

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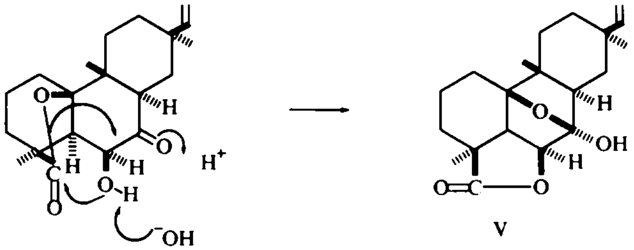
* This new metabolite (m.p. 180°) is not identical with the compound $C_{20}H_{28}O_4$ (m.p. 221°), designated Rosein III, isolated by G. G. Freeman *et al.*[7] from *T. roseum*.

† PMR spectra were recorded at 100 Mc/s for solutions in $CDCl_3$ using TMS as internal reference.

Some α-ketols, e.g. dihydrocucurbitacin B are readily autoxidized simply by exposing solutions of these compounds in ethanolic alkali to the air[11] When compound IVa was treated in this way, little autoxidation occurred and the main product was a neutral compound, $C_{20}H_{28}O_4$. This compound was not an α-ketol isomeric with IVa since it did not contain a ketone group. Thus, its UV spectrum showed no $n \rightarrow \pi^*$ ketone absorption and in the CO region its IR spectrum showed only absorption (1780 cm^{-1}) characteristic of a γ-lactone. Its CD spectrum showed a single Cotton effect at 222 mμ ($\Delta\varepsilon + 0{\cdot}66$). The new compound is formulated as V on the basis of the following additional evidence. The presence of a tertiary OH group, which absorbed at 3550 cm^{-1} in the IR region, was indicated by the stability of V to treatment with chromium trioxide in acetone. The OH proton absorbed at τ 6·20 in the PMR spectrum of V. Two doublets ($J = 6$ c/s) at τ 7·48 and τ 5·30 are ascribed to C_5—H and C_6—H, respectively. The correctness of this assignment was proved by a double resonance experiment. The C_6—O bond in V must be axial, and, since C_5—H and C_6—H clearly have the same orientation in IVa and V, it follows that the C_6—OH group in IVa must also have an axial orientation. This is in agreement with an earlier deduction.

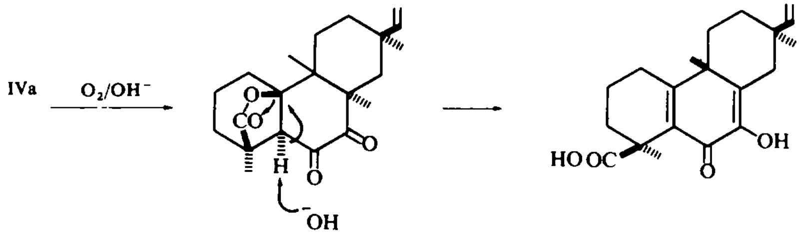
Formation of V can be rationalized on the basis of the reaction mechanism depicted in Scheme 1. This alkali-induced isomerization is surprising since rosololactone (Ia) and rosenonolactone (IIa) are recovered unchanged after similar treatment with alkali. The driving force for the reaction is probably the intramolecular hemiketal formation. The fact that V can be isolated directly from the alkaline solution indicates that the reaction proceeds in a concerted manner.

SCHEME 1



After acidification, a mixture of minor products could be extracted. The major component of this mixture, isolated by chromatography on silica, was an amorphous acid, $C_{20}H_{26}O_4$, which showed UV absorption at 256 mμ (ε 5500). The compound, for which structure VI is suggested, gave an intense purple-green ferric chloride colour reaction consistent with the presence of an enolized α-diketone system. It contained two protons which were exchanged for deuterium on treatment with deuteriomethanol. The base peak in the mass spectrum of VI occurs at *m/e* 284 due to the loss of HCOOH. The PMR spectrum showed that all the olefinic protons were contained in the vinyl group. The acid VI is probably formed by autoxidation of IVa to an α-diketone followed by opening of the lactone ring by β-elimination. The second step is analogous to the reaction of rosonolactone (Ib) with alkali.[2]

SCHEME 2



The mass spectrum (Fig. 1) of IVa shows that most of the fragments of higher mass result from the expulsion of methyl, water and CO_2 (or combinations of these) from the molecular ion which is the base peak of the spectrum. The most abundant heavy fragment ion (of mass 163) has the composition $C_{11}H_{15}O$ and is probably derived from ring C and the 7 keto group but with the loss (migration) of one hydrogen atom:

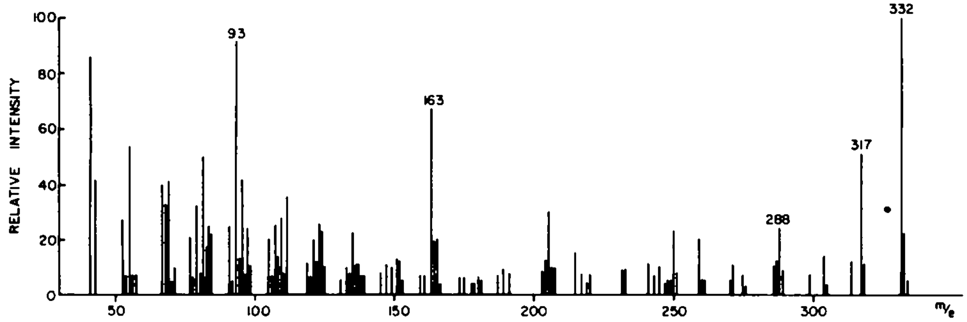


FIG. 1 Mass spectrum of 6β-hydroxyrosenonolactone.

A fragment ion of identical composition is prominent in the mass spectrum of rosenonolactone (IIa).

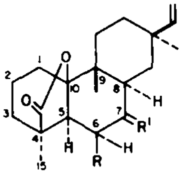
Circular dichroism of diterpene lactones

De Grazia *et al.*[12] have recently presented data on the ORD curves in the region above 210 mμ of several lactones derived from the rosane skeleton. The contribution of a substituent to the intensity of a given Cotton effect is, however, not readily determined from ORD spectra, because of the uncertain contribution of background rotation and the poor separation of different Cotton effects. We, therefore, present CD data (summarized in Table 1) on compounds Ia, Ic, IIa, IIb, IVa and IVb. All these compounds show positive lactone Cotton effects.

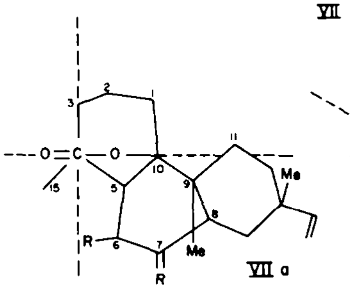
TABLE 1. CD OF LACTONES DERIVED FROM THE ROSANE SKELETON

Compound	Formula	Ketone Cotton effects		Lactone and acetate Cotton effects	
		λ (mμ)	Δε	λ (mμ)	Δε
Rosololactone	Ia			213	+3·86
Rosololactone acetate	Ic			213	+6·84
Rosenonolactone	IIa	295	−4·73	217	+3·51
Desoxyrosenonolactone	IIb			217	+3·46
6β-Hydroxyrosenonolactone	IVa	308·5	−4·58	217	+1·70
6β-Acetoxyrosenonolactone	IVb	305	−7·94	214	+7·00

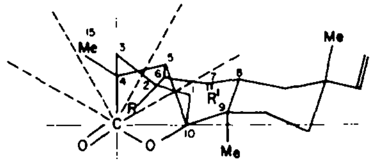
Figures VIIa and VIIb represent the octant diagrams* of diterpene lactones of the type (VII, R = H, OH or OAc; R′ = H_2 or O). On the basis of the lactone sector rule, atoms in or near the lactone sectors[13] make significant contributions to the lactone Cotton effect. In deoxorosononolactone [IIa ≡ (VIIb, R = H, R′ = H_2)], therefore, the contributions of atoms 1, 2, 5, 6 and 15 must be considered. De Grazia *et al.*[12] have argued that the contributions of atoms 5 and 15 must cancel approximately and that atoms 1 and 2, which lie in or near a positive sector (above the plane of the lactone ring), should make a bigger contribution than atom 6 which lies in a negative sector. They have therefore concluded that application of the lactone sector rule leads to the correct prediction of the lactone Cotton effect of deoxorosenonolactone (IIb), for example.



VII



VIIa



VIIb

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* The exact location of the atoms of rings B and C with respect to the sectors is slightly dependent on the nature of R and R′ since non-bonded interactions and the bond arrangements (sp^2 or sp^3) of atoms 6 and 7 affect the conformation of ring B.

The octant sector diagrams show that the oxygen function at position 7 in IIa should have only a slight effect on the lactone Cotton effect, since atom 7 lies in the region of low contribution. The Cotton effect at 217 mμ of IIa is in complete agreement with this prediction. A 6β-OH group, however, lies below the plane of the lactone ring in a negative sector. The fact that the positive lactone Cotton effect of Ia is enhanced compared with that of IIb therefore appears to contradict the prediction based on the lactone sector rule. However, Jennings *et al.*[13] have pointed out that the rules deduced for the contributions of alkyl groups may not necessarily hold for the halogen atom, hydroxyl and acetoxyl groups. In the case of Ia the possibility of intramolecular hydrogen bonding further complicates interpretation of the CD data. In view of the above findings on the effect of 6β-hydroxyl and 7-oxo groups on the rotational strength of the lactone n → π* transition, the considerable reduction in intensity of the lactone Cotton effect of IVa is unexpected. However, the introduction of a 7-keto group will slightly modify the conformation of ring B and this may greatly affect the contribution of a 6β–OH group since this substituent lies near both a sector boundary and the lactone chromophore.

The lactone and acetate Cotton effects of compound Ic are superimposed at 213 mμ. The very large increase in the intensity of this Cotton effect of Ic compared with that of the parent unsubstituted compound IIb is unlikely to be due to a high rotational strength of the acetate chromophore. Generally, an acetate chromophore has relatively low rotational strength since the group has no fixed conformation.[14] It is therefore suggested that electronic interaction between the closely situated lactone and acetate chromophores may be responsible for the much higher rotational strength of IVb compared with IVa. The very small energy separation between the chromophores increases the likelihood of electronic interaction.[15] It is possible that non-bonded and dipole–dipole interactions may cause the acetoxyl group to assume a (preferred) conformation which is skewed with respect to the lactone chromophore and also allows charge-transfer excitation. Such a situation would give rise to a high-intensity Cotton effect.[16]

The strong negative Cotton effect at 295 mμ in compound IIa is related to the ketone carbonyl absorption and may be explained in terms of a "twist" conformation of ring B.[12] The positions of the ketone CD maxima of compounds IVa and IVb compared with that of the parent compound IIa are consistent with the axial orientation of the OR (R = H or Ac) group. Most CD curves, like UV spectra, follow the rule[17] that an axial hydroxyl group shows a bathochromic shift stronger than that of an axial acetoxyl group.

The introduction of an axial OR group in the 6 position of rosenonolactone (IIa) would, on the basis of the octant rule,[18] be expected to make a positive contribution to the ketone Cotton effect and therefore cause a reduction in the intensity of the negative Cotton effect. Compound IVa shows a slight decrease in the intensity of its ketone Cotton effect. The acetate IVb, however, shows a much stronger negative Cotton effect than the parent ketone IIa. The possibility was considered that this apparent contradiction of the octant rule is due to a modified skew conformation of ring B. The change in the molecular amplitude of 2β-acetoxy and 4β-acetoxy-5α-cholestane-3-one when compared with the unsubstituted 3-one has been shown to be due to a change in conformation of ring A.[19] Inspection of Dreiding models of IVb indicate, however, that the (moderate) non-bonded interactions between the

6β-acetoxyl group and the lactone and 9β-Me groups are unlikely to be responsible for a conformational change in ring B which will result in an increased negative ketone Cotton effect. In fact, conformations of ring B which allow a decrease in the non-bonded interactions are less skew and should therefore cause a decrease in the intensity of the ketone Cotton effect. Furthermore, PMR data (*vide supra*) show that ring B has the same conformation in IVa and IVb. Factors other than distortion of the ring system must therefore be responsible for the anomalous ketone Cotton effect in compound IVb. It is suggested that electronic coupling between the acetate and lactone chromophores may be responsible. Further evidence for this electronic coupling in IVb is provided by the presence of an intense Cotton effect at 214 mμ, similar to that observed for Ic.

EXPERIMENTAL

General. UV and IR spectra were measured in MeOH and $CHCl_3$ solutions, respectively. Mass spectra were taken on a MS-9 double focusing mass spectrometer. The CD curves were measured at 20° with a Jasco ORD/UV-5 instrument with CD attachment. Concentration of solutions used for CD measurements are given in mg/ml throughout.

Isolation of 6β-*hydroxyrosenonolactone. Tricothecium roseum* Link (strain G-432) was grown on sterilized maize meal as previously described.[1] The dried, mouldy maize meal (5 kg) was extracted with 1 : 1 $CHCl_3$–MeOH and the solvent removed *in vacuo*. The toxic extract (700 g) was distributed between $CHCl_3$ (3 l.) and water (4 l.). The $CHCl_3$ was evaporated and the residue (320 g) distributed between 95% MeOH (3 l.) and hexane (5 l.). The MeOH was evaporated and the residue (50 g) filtered through Bio-Rad neutral alumina (600 g) in 9 : 1 $CHCl_3$–MeOH to remove acidic components. The filtrate was concentrated *in vacuo* to give a mixture (35 g) which was separated on neutral silica (1·3 kg). Benzene eluted deoxorosenonolactone (IIb; 600 mg), m.p. 114–116° (lit.[4] m.p. 115–116°), CD (*c*, 0·39): Δε (250 mμ) +0·23, (217) +3·46, (204) +1·54. The mass spectrum showed: M^+, 302 (calc. for $C_{20}H_{30}O_2$: M^+, 302). Elution of the column with $CHCl_3$ gave rosenonolactone (IIa; 2·2 g), m.p. 213–214° (lit.[3] m.p. 214°), CD (*c*, 0·39): Δε (350 mμ) 0, (295) −4·73, (244) 0, (217) +3·52, (208) +2·30. The mass spectrum showed: M^+, 316 (calc. for $C_{20}H_{28}O_3$: M^+, 316). Elution of the column with 99 : 1 $CHCl_3$–MeOH yielded ergosterol (800 mg), identified by direct comparison with an authentic sample.

Chloroform containing 2–4% MeOH eluted a mixture (1 g) consisting mainly of two components as shown by TLC using 25 : 1 $CHCl_3$–MeOH as mobile phase (R_f 0·35 and 0·40; spots revealed by spraying with $Ce(SO_4)_2$ in 6N H_2SO_4 and heating at 110° for 5 min). The mixture was separated by chromatography on formamide-impregnated cellulose powder (150 g). Hexane eluted rosololactone (Ia) (600 mg), m.p. 186° (lit.[2] m.p. 186°), CD (*c*, 0·40): Δε (250 mμ) +0·09, (213) +6·80, (208) +6·18. The mass spectrum showed: M^+, 318 (calc. for $C_{20}H_{30}O_3$, M^+, 318).

Elution of the formamide column with 4 : 1 hexane-C_6H_6 yielded 6β-*hydroxyrosenonolactone* (IVa; 150 mg), m.p. 178–180° (from aqueous MeOH). It showed no high-intensity absorption in the UV and had ν_{max} 3578 (sh), 3400 (br), 3070, 1768, 1718, 1638, 944 and 918 cm^{-1}, CD (*c*, 0·40): Δε (350 mμ) −0·82, (308·5) −4·58, (240) 0, (217) +1·70, (210) +0·74. [Found: C, 72·15; H, 8·50; M^+, 332·193. $C_{20}H_{28}O_4$ requires: C, 72·25; H, 8·50; M^+, 332·198].

T. roseum Link (strain G-432) was also grown on the liquid medium (3 l.) developed by Freeman *et al.*[7] for the culture of a Rosein III-producing strain of the fungus. Both the mycelia and liquid phase were extracted with $CHCl_3$ and the extracts combined. After removal of the organic solvent, the residue was chromatographed as described above. This showed that strain G-432, grown on the liquid medium, produces compounds Ia (150 mg/l.), IIa (550 mg/l.), IIb (200 mg/l.) and IVa (100 mg/l.).

Acetylation of 6β-*hydroxyrosenonolactone* (IVa). A soln of IVa (140 mg) in Ac_2O (25 ml) was cooled to −50° and 15 drops of BF_3 in Et_2O added. The mixture was allowed to come to −10° and held there for 1 hr. The reaction mixture was poured on ice and stirred for 45 min. The aqueous mixture was extracted with $CHCl_3$ and the solvent removed. Filtration of the residue in $CHCl_3$ through silica yielded the *acetate* IVb (150 mg), m.p. 179–181° (from hexane–C_6H_6), ν_{max} 1775 (sh), 1760, 1722, 1640 and 917 cm^{-1}, CD (*c*, 0·40): Δε (350) 0, (305) −7·93, (247) 0, (214) +7·00, (210) +6·54. (Found: M, 374·209. $C_{22}H_{30}O_5$ requires: M, 374·209).

Reaction of rosololactone (Ia) with Ac_2O in the presence of BF_3 and crystallization of the product from hexane yielded the acetate Ic, m.p. 166° (lit.[2] m.p. 167°), CD (*c*, 0·40): $\Delta\varepsilon$ (250 mμ) +0·09, (213) +6·80, (208) +6·18. (Found: M^+, 360. Calc. for $C_{22}H_{32}O_4$: M^+, 360).

Reaction of 6β-*acetoxyrosenonolactone* (IVb) *with* Zn *in acetic acid.* Activated Zn (600 mg) was added to a soln of IVb in HAc (15 ml). The mixture was stirred and heated under reflux for 24 hr, filtered and the filtrate concentrated *in vacuo*. The residue was taken up in $CHCl_3$, the solution washed with water and the organic phase evaporated. The residue was taken up in $CHCl_3$ and filtered through silica. The eluate contained material (30 mg) which, after crystallization from MeOH, yielded rosenonolactone (IIa), identified by m.p., mixed m.p., IR and mass spectra.

Isomerization of 6β-*hydroxyrosenonolactone* (IVa). A soln of IVa (150 mg) in 0·1N NaOH in 90% EtOH (175 ml) was allowed to stand at room temp for 4 hr. Dilution with water and extraction with CH_2Cl_2 yielded the *γ-lactone* V (121 mg), m.p. 162–163° (from Et_2O), no high-intensity absorption in the UV, ν_{max} 3550 and 1780 cm^{-1}, CD (*c*, 0·40): $\Delta\varepsilon$ (250 mμ) 0, (222) +0·66, (212) +0·12. [Found: M, 332·196. $C_{20}H_{28}O_4$ requires: M, 332·198]. The compound was recovered unchanged after treatment with chromium trioxide (2 moles) in acetone for 10 min.

Acidification (HCl) of the above aqueous phase and extraction with $CHCl_3$ yielded material (28 mg) which was chromatographed on silica (5 g). Elution with chloroform yielded mixed fractions (10 mg). Elution with 9:1 $CHCl_3$–MeOH furnished the amorphous *acid* VI, moving as one spot when examined by TLC and taken as substantially pure. It had λ_{max} 256 mμ (ε 5500), ν_{max} 3500–2400, 1705 (br), 1625 and 917 cm^{-1}. (Found: M^+, 330·184. $C_{20}H_{26}O_4$ requires: M^+, 330·183). The compound was soluble in aqueous $NaHCO_3$ and gave an intense purple-green ferric chloride colour reaction.

Acknowledgement—The authors wish to express their thanks to the South African Oil Seeds Control Board, Pretoria, for financial support.

REFERENCES

[1] De B. Scott, *Mycopathol. et Mycol. Appl.* **25**, 213 (1965).

[2] A. Harris, A. Robertson and W. B. Whalley, *J. Chem. Soc.* 1807 (1958).

[3] A. Harris, A. Robertson and W. B. Whalley, *J. Chem. Soc.* 1799 (1958); G. A. Ellestad, B. Green, A. Harris, W. B. Whalley and H. Smith, *J. Chem. Soc.* 7246 (1965) and refs therein.

[4] W. B. Whalley, B. Green, D. Arigoni, J. J. Britt and C. Djerassi, *J. Am. Chem. Soc.* **81**, 5520 (1959).

[5] A. I. Scott, D. W. Young, S. A. Hutchinson and N. S. Bhacca, *Tetrahedron Letters* 849 (1964).

[6] A. I. Scott, S. A. Sutherland, D. W. Young, L. Guglielmetti, D. Arigoni and G. A. Sim, *Proc. Chem. Soc.* 19 (1964); C. Djerassi, B. Green, W. B. Whalley and C. G. De Grazia, *J. Chem. Soc.* (C) 624 (1966) and refs therein.

[7] G. G. Freeman, R. I. Morrison and S. E. Michael, *Biochem. J.* **45**, 191 (1949).

[8] cf. R. S. Rosenfeld and T. F. Gallagher, *J. Am. Chem. Soc.* **77**, 4367 (1955).

[9] N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy to Organic Chemistry* p. 51, Holden-Day, San Francisco (1964).

[10] R. J. Abraham and J. S. E. Holker, *J. Chem. Soc.* 806 (1963).

[11] A. Melera and C. R. Noller, *J. Org. Chem.* **26**, 1213 (1961).

[12] C. G. De Grazia, W. Klyne, P. M. Scopes, D. R. Sparrow and W. B. Whalley, *J. Chem. Soc.* (C) 896 (1966).

[13] J. P. Jennings, W. Klyne and P. M. Scopes, *J. Chem. Soc.* 7211 (1965).

[14] J. P. Jennings, W. P. Mose and P. M. Scopes, *J. Chem. Soc.* (C), 1102 (1967).

[15] cf. R. C. Cookson, *Proc. Roy. Soc.* (A) **297**, No. 1448, 27 (1967).

[16] cf. N. Finch, W. I. Taylor, T. R. Emerson, W. Klyne and R. J. Swan, *Tetrahedron* **22**, 1327 (1966).

[17] R. C. Cookson, *J. Chem. Soc.* 282 (1954); R. C. Cookson and S. H. Dandegaonker, *Ibid.* 352 (1955).

[18] W. Moffit, R. B. Woodward, A. Moscowitz, W. Klyne and C. Djerassi, *J. Am. Chem. Soc.* **83**, 4013 (1961).

[19] P. Crabbé, *Optical Rotatory Dispersion and Circular Dichroism* p. 107. Holden-Day, San Francisco (1965).