The Structures of Virescenol A and B, Metabolites of *Oospora virescens* (Link) Wallr.

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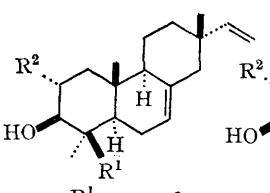
WE have already reported[1] the first results on the elucidation of the structures of virescenol A, $C_{20}H_{32}O_3$, and virescenol B, $C_{20}H_{32}O_2$, two new tricyclic diterpenes occurring as glycosides among the metabolites of *Oospora virescens* (Link) Wallr. Virescenol A was found to contain the following structural elements: 3 (—C(—)—CH_3), (—C(—)—$CH{=}CH_2$), —CH(OH)—CH(OH)—, —C(—)—CH_2OH, and —C(—)=C(—)—CH_2—. Virescenol B seemed to differ from virescenol A by the presence of only one secondary hydroxy-group.

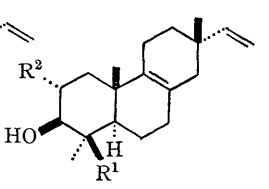
Additional work shows that the naturally occurring aglycones, virescenol A and B, have a trisubstituted double bond and can be represented by the structures (I) and (II), whereas the compounds previously[1] described were products of isomerization formed during the acidic hydrolysis, and therefore are now named isovirescenol A (V) and isovirescenol B (VI).

Virescenol A (I), $C_{20}H_{32}O_3$ (M^+ 320), m.p. 149—150°, $[\alpha]_D$ −44°,† and virescenol B (II), $C_{20}H_{32}O_2$ (M^+ 304), m.p. 146—147°, $[\alpha]_D$ −25°, can be isolated only on very mild acidic hydrolysis of the naturally occurring glycides. While the t.l.c. behaviour of the aglycones in a variety of solvent systems is the same as that of the iso-derivatives (V) and (VI), respectively, their n.m.r. spectra‡ differ from those of the $\Delta^{8,9}$-isomers (V) and (VI), mainly by the presence of an unresolved one-proton triplet centred at δ 5·38 p.p.m. (olefinic proton) and by the position of the methyl signals (virescenol A: singlets at δ 0·85, 0·9, and 1·27; virescenol B: singlets at δ 0·82, 0·85, and 1·24 p.p.m.).

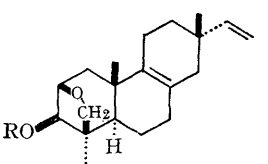
Virescenol A (I) and virescenol B (II) readily undergo an acid-catalysed isomerization to afford isovirescenol A (V) and B (VI), respectively. Treatment of isovirescenol A (V) with a 1·1 molar equivalent of toluene-*p*-sulphonyl chloride under controlled conditions leads to recovery of some starting material and the isolation of three compounds, the ditoluene-*p*-sulphonate (VII), $C_{34}H_{44}O_7S_2$ (M^+ — TsOH at *m/e* 456), the monotoluene-*p*-sulphonate (VIII), $C_{27}H_{38}O_5S$ (M^+ 474), and the ether (XIV), $C_{20}H_{30}O_2$ (M^+ 302); the structures of all three were deduced from n.m.r. evidence. While the ditoluene-*p*-sulphonate (VII) shows an oxymethylene signal (broad singlet at δ 4·07) as well as a H-2 signal (multiplet at δ 4·6 p.p.m.) downfield from those of isovirescenol A, the monotoluene-*p*-sulphonate (VIII) shows



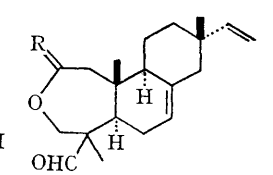
	R^1	R^2
(I)	CH_2OH	OH
(II)	CH_2OH	H
(III)	CH_2OTs	OTs
(IV)	Me	H



	R^1	R^2
(V)	CH_2OH	OH
(VI)	CH_2OH	H
(VII)	CH_2OTs	OTs
(VIII)	CH_2OTs	OH
(IX)	Me	OH
(X)	CH_2OTs	H
(XI)	Me	H
(XII)	Me	H,$\Delta^{8,14}$
(XIII)	Me	H,3-oxo



(XIV) R=H
(XV) R=Ac



(XVI) R=H, OH
(XVII) R=O

only the former signal (δ 4·13) downfield. In both compounds H-3 gives rise to a doublet centred at δ 3·25 and 3·00 p.p.m. respectively, the large coupling constant (*J* 10 c./sec.) indicating a diaxial H-2, H-3 configuration. In the ether (XIV), H-3, now adjacent to a H-2 equatorial proton, gives a singlet at δ 3·46 p.p.m. [shifted to δ 4·73 in its acetate (XV)]. Formation of this ether must have occurred by way of the intermediacy of a 2α-toluene-*p*-sulphonate and internal displacement of this by the primary hydroxy-group. This forces

the latter to be part of an axial hydroxymethyl group, in agreement with its characteristic chemical shift.[1,2]

Lithium aluminium hydride reduction of the monotoluene-*p*-sulphonate (VIII) gives the glycol (IX), $C_{20}H_{32}O_2$ (M^+ 304), m.p. 98—100°, $[\alpha]_D$ +61°. Similar reduction of the ditoluene-*p*-sulphonate (VII) affords the mono-alcohol (XI), $C_{20}H_{32}O$ (M^+ 288), m.p. 106—108°, $[\alpha]_D$ + 92° [methyl signals: two (3H) singlets at δ 0·81 and 1·03 and one (6H) singlet at δ 0·98; H-3: multiplet centred at δ 3·25 p.p.m.]. Partial toluenesulphonation of isovirescenol B (VI) leads to the monotoluene-*p*-sulphonate (X), lithium aluminium hydride reduction of which yields the mono-alcohol (XI) also. This compound is identical with the product of mild acid-catalysed isomerization[3] of sandaracopimaradien-3β-ol[4] (XII). Furthermore, the three samples of alcohol (XI), derived from isovirescenol A (V) and B (VI) and 8,9-sandaracopimaradien-3β-ol, yielded the same ketone (XIII), m.p. 48—50°, $[\alpha]_D$ + 154°, on Jones oxidation.

These findings suggest 8,14 or 7,8 as the location of the trisubstituted double bond of the naturally occurring virescenols. The following facts support the latter choice: (a) the n.m.r. signal shape of the 13α-vinyl group, characteristic and different for 7,8, 8,14, and 8,9 nuclear double bond systems,[5] indicates an isopimaric structure for the natural alcohols; the signal shape of the hydrogen of the nuclear double bond is even more striking and comparable to that of isopimaric systems;[5] (b) lithium aluminium hydride reduction of the toluene-*p*-sulphonate (III) of natural virescenol A affords the mono-alcohol (IV), $C_{20}H_{32}O$, m.p. 128—130°, $[\alpha]_D$ − 28° [Me signals: two (3H) singlets at δ 0·98 and 1·00 and one (6H) singlet at δ 0·88; H-7: unresolved 1H triplet centred at δ 5·36 p.p.m.], different from sandaracopimaradien-3β-ol (XII).

Periodate oxydation of virescenol A (I) yields an aldehydo-hemiacetal (XVI), $C_{20}H_{30}O_3$ (M^+ 318), m.p. 118—120°, $[\alpha]_D$ + 61° [aldehydic proton: singlet at δ 9·38; H-2 and H-7: 2H multiplet centred at δ 5·31; oxymethylene: doublets centred at δ 3·15 and 4·23 p.p.m. (J 12·5 c./sec.)], Fétizon oxidation[6] of which leads to an aldehydo-lactone (XVII), $C_{20}H_{28}O_3$ (M^+ 316), m.p. 129—131°, ν(CO) 1720 and 1740 cm.$^{-1}$ [aldehydic proton: singlet at δ 9·47; oxymethylene: doublets centred at 3·88 and 4·33 (J 12 c./sec.); H-1: 2H singlet at δ 2·72 p.p.m.]. The spectral properties of the latter are consistent with the presence of a C-4 axial hydroxymethyl group in virescenol A and excludes its alternative location at C-10.

From these results virescenol A and virescenol B can be considered as isopimaradien-2α,3β,19-triol and isopimaradien-3β,19-diol, respectively. Virescenol A represents the first ring A tri-oxygenated diterpenic fungal metabolite. A study of the sugar moieties of the glycosides is in progress.

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† All the optical rotations were measured in $CHCl_3$.
‡ The n.m.r. spectra were measured in $CDCl_3$.

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