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MINOR TERPENOIDS OF *TRICOTHECIUM ROSEUM*

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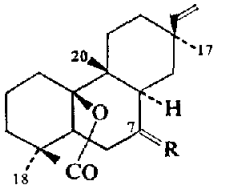
Abstract—The isolation of rosenololactone (I) and crotocin (VI) from *Tricothecium roseum* is described.

DURING a study[1] of the biosynthesis of the terpenoid metabolites of *Tricothecium roseum*, the constituents of the broth and mycelium were examined. This has led to the isolation of a number of new metabolites, some of which are described in this paper.

When *T. roseum* (strain CM1 50,660) was grown on surface culture on a Czapek-Dox-ammonium tartrate: C.S.L. medium for 4 weeks, the major metabolites were rosenonolactone (II) and rosololactone.[2] Desoxyrosenonolactone (III),[3] 6β-hydroxyrosenolactone[4] and tricothecin[5] were isolated in smaller amounts. Occasionally the fermentation produced quantities of ergosterol and very little of the normal metabolites. Rosein III[6] and isorosenolic acid[7] were not encountered.



Rosenololactone (I), R = 

Rosenonolactone (II), R = O

Desoxyrosenonolactone (III), R = H_2

Chromatography of the neutral extract on alumina has now given a new metabolite, $C_{20}H_{30}O_3$, m.p. 219–221° (*m/e* 318), which is isomeric with rosololactone. Its i.r. spectrum enabled the oxygen functions to be accounted for as γ-lactone (ν_{max} 1750 cm^{-1}) and hydroxyl (3500 cm^{-1}) whilst olefinic absorption at 1650 and 940cm^{-1} indicated a vinyl group.

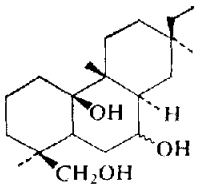
[1] B. ACHILLADELIS and J. R. HANSON, *Phytochem.* 7, 589 (1968); *Tetrahedron Letters* 4397 (1968).
[2] A. ROBERTSON, W. R. SMITHIES and E. TITTENSOR, *J. Chem. Soc.* 879 (1949);
A. HARRIS, A. ROBERTSON and W. B. WHALLEY, *J. Chem. Soc.* 1799 (1958).
[3] W. B. WHALLEY, B. GREEN, D. ARIGONI, J. J. BRITT and C. DJERASSI, *J. Am. Chem. Soc.* **81**, 5520 (1959).
[4] C. W. HOLZAPFEL and P. S. STEYN, *Tetrahedron* **34**, 3321 (1968);
A. J. ALLISON, J. D. CONNOLLY and K. H. OVERTON, *J. Chem. Soc.* 2122 (1968).
[5] G. G. FREEMAN, J. E. GILL and W. S. WARING, *J. Chem. Soc.* 1105 (1959);
J. FISHMAN, E. R. H. JONES, G. LOWE and M. C. WHITING, *J. Chem. Soc.* 3948 (1960).
[6] G. G. FREEMAN, R. I. MORRISON and S. E. MICHAEL, *Biochem. J.* **45**, 191 (1949).
[7] A. I. SCOTT, D. W. YOUNG, S. A. HUTCHINSON and N. S. BHACCA, *Tetrahedron Letters* 849 (1964).

The NMR spectrum showed resonances at τ 4·12, 4·95, 5·20 characteristic of a vinyl group, a single proton multiplet at 6·10 (CH.OH) and three C-methyl groups (see Table 1). Catalytic reduction gave a dihydro compound which formed a monoacetate. The metabolite proved to be identical with rosenololactone, the $NaBH_4$ reduction product of rosenonolactone (II). The 7-hydroxyl group was assigned a β-configuration. Approach to the β-face of ring B is hindered by the 9β-methyl and lactone substituents and hence attack of a hydride reagent is directed to the α-face of ring B, borohydride reduction of a 6-ketone is known to give a 6β-alcohol.[8] Hence $NaBH_4$ reduction of rosenonolactone was expected to give a 7β-alcohol. Approach of the α-face of the molecule to a catalyst surface is favoured for the same reason. Catalytic reduction of rosenonolactone led to dihydrorosenololactone. The 7β-hydroxyl lies on the same side of the molecule as the 9β-methyl group. This is reflected in solvent shifts of the methyl groups in the NMR spectra of rosenololactone and its relatives (see Table 1).

TABLE 1. C-METHYL RESONANCES OF *Tricothecium* DITERPENOIDS IN DEUTEROCHLOROFORM AND DEUTEROPYRIDINE

	C-17		C-18		C-20	
	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N
Rosenololactone	9·00	8·96	8·89	8·88	8·89	8·66
Dihydrorosenololactone	9·14	9·12	8·88	8·88	8·88	8·68
Desoxyrosenonolactone	9·04	9·05	9·04	9·00	8·92	8·92
Rosenonolactone	9·08	9·08	9·04	9·03	8·90	8·90

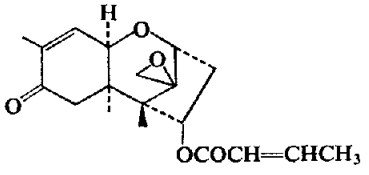
Attempts to obtain chemical confirmation of this by oxidation of dihydrorosenololactone to a 7-20 ether with $Pb(OAc)_4$ afforded dihydrorosenonolactone as the sole crystalline product. $LiAlH_4$ reduction of dihydrorosenololactone gave Triol A (IV).[9] This has been assigned an equatorial 7α-hydroxyl group on the basis of the facile hydrolysis of its acetate. However this result is equally compatible with an axial 7β-hydroxyl group in which anchimeric assistance to hydrolysis is provided by a transannular interaction with the 10-hydroxyl group. Such reversal of the equatorial : axial order of hydrolysis is known in other instances in which a hydroxyl group is suitably orientated[10] to provide assistance. The implication of rosenololactone in the biosynthesis of rosenonolactone will be discussed elsewhere.



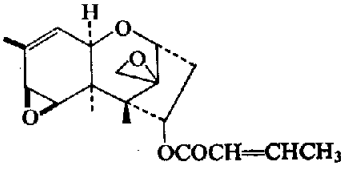
Triol A (IV)

[8] M. R. COX, G. A. ELLESTAD, A. J. HANNAFORD, I. R. WALLWORK, W. B. WHALLEY and B. SJOBERG, *J. Chem. Soc.* 7257 (1965).
[9] G. A. ELLESTAD, B. GREEN, A. HARRIS, W. B. WHALLEY and H. SMITH, *J. Chem. Soc.* 7246 (1965).
[10] H. B. HENBEST and B. J. LOVELL, *J. Chem. Soc.* 1965 (1957).

The second new metabolite isolated from the fermentation gave analytical data for $C_{19}H_{24}O_5$. It was thus isomeric with tricothecin (V) to which it showed marked similarities in the i.r. spectrum, lacking however the carbonyl absorption at 1675 cm^{-1}. The NMR spectrum revealed the presence of the crotonyl side-chain but showed major differences in the τ 6·5–7·3 region. The metabolite proved to be identical with crotocin (antibiotic T) (VI),[11] a sample of which was generously supplied by Dr. Gyimesi. Tricothecolone, the sesquiterpenoid from which tricothecin is derived, was also isolated from the fermentation.



Tricothecin (V)



Crotocin (VI)

EXPERIMENTAL

Isolation of Metabolites

Tricothecium roseum (CMI 50,660) (15 l.) was cultured, harvested and extracted as previously described.[1] The petrol ether soluble portion of the mycelium extract was absorbed on silica gel and chromatographed on Al_2O_3 (grade II). Elution with benzene yielded desoxyrosenonolactone whilst 1% ether:benzene yielded tricothecin. Elution with 5% ether:benzene yielded rosenololactone (24·3 mg, 1·6mg/l) as prisms, m.p. 218–221° (lit.[2] 222°), $[\alpha]_D + 4°$ (Found: C, 75·2; H, 9·6. Calc. for $C_{20}H_{30}O_3$: C, 75·4; H, 9·5 per cent), ν_{max} 3520, 1755, 1645, 960, 920 cm^{-1}. The compound was identical (mixed m.p., TLC, i.r. and NMR) with a sample of rosenololactone prepared by the reduction of rosenonolactone with $NaBH_4$ in methanol. Catalytic hydrogenation gave dihydrorosenololactone, m.p. 195–196° (lit.[9] 196°). The *acetate* of dihydrorosenololactone, prepared with acetic anhydride, sodium acetate had m.p. 168–169° (Found: C, 73·1; H, 9·4. $C_{22}H_{34}O_4$ required: C, 72·9; H, 9·45 per cent), ν_{max} 1770 and 1735 cm^{-1}, τ 9·18 (3), 8·92 (6), 7·98 (3), 4·98 (1).

The $CHCl_3$ extract of the broth, after removal of the solvent, was absorbed on silica and chromatographed on Al_2O_3 (grade II). Elution with 1% ether:benzene yielded crotocin (14·3 mg, 0·9 mg/l) as needles, m.p. 116–118°, ν_{max} 1710, 1640 cm^{-1}; i.r. identical to that of a sample provided by Dr. Gyimesi. Subsequent fractions gave rosenonolactone and rosololactone. Elution with 20% ether:benzene gave 6β-hydroxyrosenonolactone (43·5 mg, 2·8 mg/l), m.p. 180–181° (lit.[4] 178–180). Elution with 3% methanol:ether gave tricothecolone (52 mg, 3·5 mg/l).

Catalytic Reduction of Rosenonolactone

Rosenonolactone (200 ml) in acetic acid (10 ml) containing $HClO_4$ (0·1 ml) was hydrogenated over Adams' catalyst (50 mg) until uptake ceased. Recovery gave dihydrorosenololactone (94 mg) as needles, m.p. 195–196° (lit.[9] 196°), ν_{max} 3530 and 1755 cm^{-1}, i.r. spectrum identical to a sample prepared by the hydrogenation of rosenonolactone over 10% Pd/C.

Lead Tetraacetate Oxidation of Dihydrorosenololactone

Lead tetraacetate (200 mg) and $CaCO_3$ (100 mg) were heated under reflux in A.R. benzene (15 ml) for 15 min. The lactone (84 mg) was then added and refluxing continued for a further 3 hr. Recovery and chromatography on Al_2O_3 gave, in the fractions eluted with 10% EtAc:petrol ether, dihydrorosenonolactone (34 mg), m.p. 180–181° (lit.[2] 181–182°, ν_{max} 1775 and 1715 cm^{-1}); i.r. spectrum identical to a sample prepared by the hydrogenation of rosenonolactone over 10% Pd/C. Comparable results were obtained with iodine, lead tetraacetate and irradiation with u.v. light.

Lithium Aluminium Hydride Reduction of Dihydrorosenololactone

The lactone (120 mg) in ether (15 ml) was treated with $LiAlH_4$ (250 mg) for 24 hr. Recovery gave the triol A, m.p. 143–145° (lit.[9] 146°), ν_{max} 3500 cm^{-1} (br).

[11] J. GYIMESI and A. MELERA, *Tetrahedron Letters* 1665 (1967).