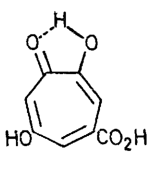
Determination of the Structure of Stipitalide, a New Tropolone from *Penicillium stipitatum* Thom

By MIROSLAV HOLÍK* and IVO KUHR

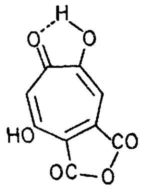
(*Research Institute of Pure Chemicals, Lachema, Brno and Research Institute of Veterinary Medicine, Brno, Czechoslovakia*)

Summary The structure of 6-hydroxy-4-hydroxymethyltropolone-5-carboxylic acid γ-lactone was established for a new tropolone metabolite.

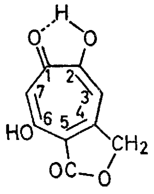
SEVERAL years ago three fungal tropolones of *Penicillium stipitatum* Thom were discovered: stipitatic acid (I),[1] stipitatonic acid (II),[2] and ethyl stipitate.[3] Now, a new tropolone derivative, stipitalide (III), has been isolated.[4] Similar γ-lactone-ring compounds were found in some fungal and lichen metabolites.[5]



(I)



(II)



(III)

It is believed that stipitalide is a precursor of stipitatonic acid in its biosynthesis *via* 3-methylorsellinic acid[6]. The amount of stipitalide in the filtrate of a shaken culture of *P. stipitatum* CBS is greatest after 72—92 h of cultivation while the maximum yields of stipitatonic acid were obtained after 92—120 h. Detailed experimental conditions and the isolation of (III) are described elsewhere.[4]

TABLE. *N.m.r. signals of the tropolones* (I)—(III) (τ)[a]

	7-H	3-H	5-H	CH_2
(I)	3·17 d (2·5)[b]	2·68 d (1·5)[b]	2·58 dd (2·5; 1·5)[b]	—
(II)	3·21 s (1·4)[c]	3·05 s (1·5)[c]	—	—
(III)	3·18 s (1·7)[c]	3·13 s (2·5)[c]	—	4·74 s (2·2)[c]

[a] In $(CD_3)_2SO$ against Me_4Si internal standard; [b] long-range coupling constant ^4J in Hz; [c] line width at half height, $w_{\frac{1}{2}}$, in Hz.

Besides the distinct bands characteristic for tropolones[7] (1260, 1560, and 1622 cm^{-1}) the i.r. spectrum of stipitalide shows an additional carbonyl absorption at 1717 cm^{-1}. The m.s. reveals M 194 and the molecular formula $C_9H_6O_5$. Also the mass spectral fragmentation with loss of three CO groups, confirmed by the presence of the corresponding metastable ions, testifies to the structure of a carbonyl-substituted tropolone.

The structural relationship of stipitalide to stipitatonic and stipitatic acids was also confirmed by n.m.r. spectroscopy (see Table). 3-H and 7-H in compounds (I)—(III) do not show any observable mutual spin–spin coupling and the chemical shift of 7-H is nearly the same for all three. Therefore the mutual position of 7-H and 3-H in the ring must be the same in (I)—(III) and the structures differ only in the surroundings of the 3-H proton. The carboxy-group in (I) decreases the chemical shift of 3-H while the alkyl group in (III) has the opposite effect. The observed chemical shift of the methylene group of (III), τ 4·74, compares favourably with that calculated from the Schoolery rule, τ 4·81. The 3-H and CH$_2$ singlets are broadened ($w_{\frac{1}{2}} = 2{\cdot}5$ and 2·2 Hz, respectively) due to long range coupling. Double resonance involving irradiation of the methylene signal causes narrowing of the 3-H singlet ($w_{\frac{1}{2}} = 1{\cdot}7$ Hz) and enhancement of its amplitude, while the 7-H signal remains unchanged. In this manner the other possible isomer of stipitalide, 6-hydroxy-5-hydroxymethyltropolone-4-carboxylic acid γ-lactone, can be excluded.

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[1] J. H. Birkinshaw, A. R. Chambers, and H. Raistrick, *Biochem. J.*, 1942, **36**, 242.
[2] W. Segal, *J. Chem. Soc.*, 1959, 2847.
[3] P. V. Divekar, P. E. Brenneisen, and S. W. Tanenbaum, *Biochim. Biophys. Acta*, 1961, **50**, 588.
[4] I. Kuhr and J. Fuska, *Appl. Microbiol.*, in the press.
[5] W. B. Turner 'Fungal Metabolites,' Academic Press, London, 1971.
[6] A. I. Scott, H. Guilford, and E. Lee, *J. Amer. Chem. Soc.*, 1971, **93**, 3534 and references therein.
[7] G. Aulin-Erdtman and H. Theorell, *Acta Chem. Scand.*, 1950, **4**, 1490.