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OXYSPORONE, A NEW METABOLITE FROM *FUSARIUM OXYSPORUM*

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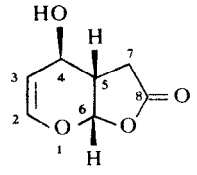
Key Word Index—*Fusarium oxysporum*; mould; oxysporone; fusaric acid; dehydrofusaric acid.

A finely ground mixture of earthworm casts and salt to taste is used in some parts of Nigeria to treat chronic dysentry. (D. S. Adoun, former Headmaster of E.K.A. gave us this anecdote. His mother experienced rapid cures by its use; he has since been using it with consistently good results). On the assumption that the alleged activity was in the casts, we decided to investigate the antibiosis of earthworm casts (collected in 7 Laird Place, University of Ibadan) by looking at secondary metabolites from its microorganisms. We have been able to establish antibiotic activity in the casts as a whole and found that the activity was dependent on the presence of living microorganisms within the casts (Alo, B. I. and Adesogan, E. K., unpublished results). One of the fungi isolated was *Fusarium oxysporum* var. Schelecht (accession No. IMI 211881 at the Commonwealth Mycological Institute). On Czapek-Dox medium, there was no pigmentation and the fungus gave fusaric acid and its dehydro-analogue. The fungus pigmented heavily however on Raulin–Thom medium. Most of the pigments on the fourth day was due to non-volatile naphthazarins [1]. On the eighth day, a red oil was isolated from which oxysporone was separated (*ca* 15 mg per l. culture fluid—pure by GLC) by elution with ethyl acetate–petrol (2:3) on a Si gel column.



1

We propose the structure **1** for oxysporone on the basis of the following spectroscopic and chemical evidence. Oxysporone had IR absorptions at $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1806 (γ-alkoxy-γ-lactone [2]; cf. [3–5]), 1654 (C=C) and UV absorptions at λ_{max} nm: 215 (ε 1277) and 284 (ε 417). These assignments were in agreement with the NMR spectra of **1**. The ^{1}H NMR spectrum (100 MHz, δ) exhibited signals at 6.37 (1H, *d*, J = 6Hz, H2); 5.06 (1H, *ddd*, J = 6, 5.5, 1.0 Hz, H-3 coupled to H-2, H-4 and one of the high field protons possibly H-5); 5.82 (1H, *d*, J = 4.5 Hz, H-6); 4.16 (1H, *dd*, J = 5.5 and 2.0 Hz, H-4) and four high field protons between δ2 and 3, one of which was OH (exchange with D_2O). The ^{13}C NMR spectrum had absorptions at δ 175.3 (C-8), 143.5 (C-2), 100.1 (C-3), 96.0 (C-6), 60.0 (C-4), 41.8 (C-7) and 29.5 (C-5). The high resolution electron impact MS gave an M^+ ion at *m/e* 156.042, $C_7H_8O_4$. The major peaks in the MS at *m/e* (ret. int.) 43 (100, $[CH_2CHO]^+$); 84 (100; $[C_4H_4O_2]^+$. $-\beta\gamma$-unsaturated γ-lactone radical cation); 73 (87, $[CHO - CH_2 - CH_2O]^+$) and 55 (64), were attributed principally to a retro-Diels–Alder fragmentation, with the *m/e* 43 ion conceivably arising from α-cleavage of the malonyl dialdehyde and the *m/e* 73 ion by H-transfer to the dialdehyde, while the fragment at *m/e* 55 could arise by the loss of CHO from the *m/e* 84 fragment ion.

Oxysporone formed a monoacetate, $\nu_{max}^{CHCl_3}$ cm^{-1}: 1807 (γ-alkoxy-γ-lactone), 1738 (ester) and 1656 (C=C), whose ^{1}H NMR spectrum showed that the proton formerly at 4.16 had shifted to 5.08. On hydrogenation over Pt at NTP, oxysporone lost the IR absorption at ν_{max} 1654 cm^{-1}, but that at 1806 was not shifted.

In view of the fact that H-2 was a clean doublet and therefore not allylically coupled to H-4, we infer that the conformation of the molecule is one that gives them an allylic angle, of zero or almost zero. Furthermore, the 2 Hz coupling constant between H-4 and H-5 was indicative of a dihedral angle of *ca* 90° between these two protons. The Dreiding model of oxysporone, which incorporates these facts as well as giving a dihedral angle which is consistent with the coupling constants between H-5 and H-6, gives the structure shown in **1**. Oxysporone is therefore assigned the structure and relative stereochemistry shown in **1**.

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VOLATILE CONSTITUENTS OF *LEPECHINIA CALYCINA*

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Key Word Index—*Lepechinia calycina*; Labiatae; monoterpenes; δ-3-carene; essential oil.

In a previous report by one of us, the chemical composition of the essential oil of a native North American Labiatae, *Satureja douglasii*, was described [1]. In this note, we report the chemical composition of an essential oil of another native North American Labiatae, *Lepechinia calycina* Epl. Using techniques that have been described previously [2], the oil of *L. calycina* was found to contain 1,8-cineole (19.7%), camphor (17.5%), δ-3-carene (17.4%), camphene (7.8%), α-pinene (6.5%), caryophyllene (5.7%), linalool (2.6%), α-terpinene (2.3%), limonene (2.3%), β-pinene (1.8%), *trans*-nerolidol (1.8%), myrcene (1.7%), α-terpineol (1.5%), γ-terpinene (1.4%), α-phellandrene (1.0%), terpinolene (0.9%), piperitone (0.8%), δ-cadinene (0.8%), *p*-cymene (0.7%), terpinene-4-ol (0.7%), α-phellandrene (0.6%), menthone (0.6%), phenylethyl butyrate (0.6%), *trans*-β-farnesene (0.3%), 10-epi-α-cadinol (0.3%), α-thujene (0.2%), isomenthone (0.2%), α-gurjunene (0.2%), α-humulene (0.2%), bornyl acetate (0.1%), geranyl acetate (0.1%) and trace amounts of neoisopulegol and phenylethyl 2-methyl butyrate.

From a comparative standpoint, the chemical composition of *L. calycina* is not dissimilar to that found in other North American Labiatae. For example, in 1967 Emboden and Lewis [3] showed that some 15 species of *Salvia* contained 1,8-cineole as one of the major components. In addition, with the exception of *Salvia columbariae*, *S. pachyphylla* and *S. brandegei*, all of the other species were found to contain camphor as one of the other major components.

The occurrence of δ-3-carene in the oil of *L. calycina* is somewhat unusual, as it was not found as a constituent of the aforementioned *Salvia* species. It is more normal to find δ-3-carene as a constituent of the Pinaceae, not the Labiatae; its occurrence in *Lepechinia* may thus be of chemotaxonomic interest.

EXPERIMENTAL

Plant material was obtained from University of California Botanical Gardens. A specimen of this plant has been placed in the herbarium at University of Waterloo. The essential oil used in the analysis was obtained using a modified Clevenger apparatus. All individual compounds were identified by careful comparison of their IR spectra with those of authentic markers. Percentage composition measurements were made with the aid of electronic integration of a flame detection from a capillary GLC run.

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