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FRUSTULOSINOL, AN ANTIBIOTIC METABOLITE OF *STEREUM FRUSTULOSUM*: REVISED STRUCTURE OF FRUSTULOSIN

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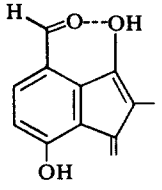
Key Word Index—*Stereum frustulosum*; Basidiomycete; fungal metabolite; naturally-occurring acetylene; frustulosinol; frustulosin.

Abstract—On the basis of chemical reactions and spectroscopic data, frustulosinol, a new antibiotic metabolite of *Stereum frustulosum*, has been assigned an acetylenic structure. This has led to a revised structure for the related metabolite, frustulosin.

In a preliminary communication [1] we proposed the structure **1** for frustulosin, an antibiotic metabolite of *Stereum frustulosum*. The compound was available only in microquantities, and therefore, the structure was arrived at entirely on the basis of spectroscopic evidence. With the object of obtaining larger amounts of frustulosin for biological screening, we examined several other strains of this fungus. In the course of this search we detected a second metabolite, frustulosinol, which we were able to isolate in substantial amounts. Evidence from chemical investigation of this compound, combined with spectroscopic data, led unequivocally to a monocyclic structure with an isopentenyne side chain (**2**). Frustulosinol could be obtained from frustulosin by reduction with $NaBH_4$. Therefore, frustulosin must have the (revised) structure **3**.



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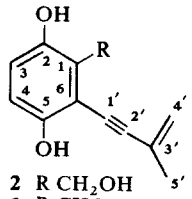
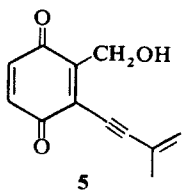


2 R CH_2OH
3 R CHO



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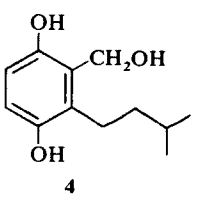

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In serial dilution tests, frustulosin was active against *Staphylococcus aureus*, *Bacillus mycoides*, and *B. subtilis* at a concentration of 16 ppm, and against several fungal species at somewhat higher concentrations. It was also moderately active against *Vibrio cholera* and *V. cholera* phage. Frustulosinol and the quinone derived from it were active against *S. aureus* at a concentration of 16 ppm and at 64–256 ppm against *Mycobacterium smegmatis*.

Frustulosinol $C_{12}H_{12}O_3$ (elemental analysis) had mp 87–89°; MW 204 (MS); λ_{max}^{EtOH} 330 (3.85), 280 (4.05), 266 (4.09), 253 sh (3.95) and 207 (4.36) nm; ν_{max} 3425, 1613, 869, 806 cm^{-1}. The PMR spectrum showed a doublet of a doublet at 2.01 (3H, *J* 1.0 and 1.6 Hz) for the methyl protons, a singlet at 5.07 (2H) for the benzylic methylene, 2 doublets of quartets at 5.40 (1H, *J* 1.6 and 2.0 Hz) and at 5.47 (1H, *J* 1.0 and 2.0 Hz) for the methylene protons, and a singlet at 6.80 (2H) for the aromatic protons. The hydroxyl protons appeared as a broad peak around 6.83 ppm. Frustulosinol formed a triacetate.

On catalytic hydrogenation, frustulosinol absorbed 3 mol hydrogen to give the hexahydro compound (**4**) mp 136–138°; MW 210 (MS); λ_{max}^{EtOH} 296 (3.63), 197 (4.26) nm; (the 296 maximum shifts as a broad peak at *ca* 275 on adding alkali); ν_{max} 3367, 3106, 1618 cm^{-1}. The PMR spectrum in pyridine *d5* showed signals at 0.96 (*d*, 6H, $J = 7$ Hz) for the *gem*-dimethyl protons, a multiplet

around 1.7 (3H) for the C-2′ and C-3′ protons, another multiplet at 3.10 (2H) for the C-1′ protons, a singlet at 5.30 (2H) for the hydroxymethylene protons and an AB quartet at 6.90 and 7.05 (J = 7 Hz) for the aromatic protons. The hydroxyl protons appeared as a broad hump around 5.90 ppm. In the acetate of the hexahydro derivative the *gem*-dimethyl protons appeared at 0.97 and the C-2′ methylene protons at 1.18 (*m*, 2H). The spectrum also showed signals at 1.34 (*m*, 1H) and 2.6 (*m*, 2H) for the C-3′ and C-1′ protons, 2.02 (*s*, 3H) and 2.30 (*s*, 6H) for the three acetates, 6.10 (*s*, 2H) for the acetoxymethylene, and an AB quartet around 6.93 and 7.03 (J = 7 Hz) for the aromatic protons. Multiplicity of the C-1′ proton signals (6 lines) is consistent with the AA′ BB′ splitting of C-1′ and C-2′ protons [2]. Thus, the PMR spectra of the hydrogenation products locate the six new protons in the side chain, and define this as 3-methyl butyl. Accordingly, frustulosinol itself must have an enyne side chain.

On treatment with Ag_2CO_3 on Celite, frustulosinol was oxidized to a quinone, **5**, mp 62–64°; MW 202 (MS) λ_{max}^{EtOH} 395 (*br*) and 250 nm; ν_{max} 1680, 1652 and 1577 cm^{-1}. The PMR spectrum showed signals at 2.01 (*dd*, J = 1.0, 1.6 Hz) for the methyl protons, 2.90 (*t*, 1H J 6 Hz) for the hydroxyl protons, 4.76 (*d*, 2H J = 6 Hz) for the hydroxymethylene protons, 5.58 (*dq* 1H J = 1.6 and 2.0 Hz) and 5.64 (*dq* 1H J = 1.0 and 2.0) for the methylene protons, and 6.86 (*s*, 2H) for the ring protons. The ultraviolet absorption spectrum of the quinone, as well as the equivalence of the ring protons in its PMR spectrum, shows that it is a *p*-quinone. Further, as in the case of hexahydrosiccayne [3] and other *p*-dihydroxy benzene derivatives, the UV maximum of hexahydro frustulosinol underwent a blue shift on adding alkali. The aromatic protons appeared as an AB quartet (J = 7 Hz) in the spectra of hexahydro frustulosinol and its acetate, and therefore, they are *ortho* to each other. Thus, the substitution pattern of frustulosinol is established as in **2**.

The ^{13}C NMR spectrum of frustulosinol showed peaks at 22.8 (C-5′) 58.5 (CH_2OH), 83.9 and 97.6 (*sp* carbons), 109.7* (C-3′), 114.9 and 117.1 (C-3 and C-4), 121.2* (C-4′) 126.8* (C-6), 128.0* (C-1), 148.4 and 151.1 (C-2 and C-5) ppm. The normal shift of sp carbons falls in the range of 65–90 ppm, [4] but this generalization is based on relatively few examples compared to the number available for sp^2 and sp^3 carbons. Structure **3** for frustulosin was not considered originally because in its ^{13}C NMR spectrum in $CDCl_3$ no peak was observed within this range. However, in the light of the structure of frustulosinol we considered the possibility that an acetylenic carbon peak might be masked by the $CDCl_3$ signals. Accordingly, we recorded the spectrum in DMSO-d_6. Under these conditions it showed peaks at 80.8 and 100.6 ppm attributable to the acetylenic carbons. In the original spectrum in $CDCl_3$ a very low intensity peak assigned to one of the olefinic carbons turned out to be spurious.

Frustulosin and frustulosinol are, to our knowledge, the only acetylenic compounds isolated from a *Stereum* species, although the detection of a polyacetylenic spectrum in fruiting bodies of *Stereum hirsutum* has been reported [5].

The biogenesis of these metabolites presents an interesting problem. Gentisyl alcohol, which differs from frustulosinol only in that it lacks the isopentyl side chain, is biosynthesized by two different routes [6]: In the Fungi Imperfecti it is formed from acetate; in the Basidiomycetes and higher plants it arises from shikimic acid. We intend to study the biogenesis of frustulosinol to determine whether the side chain arises from mevalonic acid and the remainder of the molecule is formed by the shikimic acid pathway.

* Tentative assignments.

EXPERIMENTAL

General procedure. Mps are uncorrected. Unless otherwise stated, PMR and ^{13}C NMR spectra were measured in $CDCl_3$ solution with TMS as internal standard on a Varian HR 100 and a Jeol PS-100 spectrometer, respectively, and are expressed on the δ scale. IR spectra were taken in KBr pellets on a Perkin–Elmer Model 21 spectrometer. Elemental analysis was carried out by Dr. Pascher, Bonn, Germany and Mass Spectra were recorded by Morgan–Schaffer, Corp., Montreal, Canada.

Isolation of frustulosinol and frustulosin. Stereum frustolosum† was grown in still culture in a 5% cornsteep medium in the dark, and culture liquid was collected 5 weeks after inoculation and its ethyl acetate extract was taken to dryness *in vacuo*. Residue was distributed in a 50-tube countercurrent apparatus using a pre-equilibrated 50% aqueous MeOH–petroleum ether system. Tubes 28–40 contained mainly frustulosin. This was further purified by crystallization from ethyl acetate–petrol (1:1). Frustulosin mp 139–40°, $C_{12}H_{10}O_3$ (elemental analysis) MW 202 (MS), had λ_{max}^{EtOH} 402 (3.83) 293 (3.83) 273 (*sh* 3.82) 249 (4.29) 237 (*sh* 4.17). ν_{max} 3279, 1645, 1613 and 1522 cm^{-1}, MS peaks at 202 (M^+ 100%) 187 (M-15, 20%) 174 (M-28, 20%); PMR signals at 2.10 (*dd* 3H, J = 1.0 and 2.0 Hz) a broad peak at 4.5 for the OH proton, two doublets of quartets (1H each) at 5.44 (J = 1.6 and 2.0 Hz) and 5.52 (J = 1.0 and 2.0 Hz), and an AB quartet (J = 8 Hz) at 6.88 and 7.16. The quartet was further split by long range coupling with the aldehyde and the chelated proton (J = 0.4 Hz each). The aldehyde and the chelated phenol protons appeared at 10.31 (*d*, 1H J = 0.4 Hz) and 11.22 (*d*, 1H J = 0.4 Hz). The ^{13}C NMR spectrum in DMSO-d6 showed peaks at 22.8 (Me), 80.8 and 100.6 (acetylenic carbons) 110.56* (C-3′), 118.4* (C-4′), 118.7 (C-6), 122.6, 125.2 (C-3 and C-4), 126.1 (C-1), 151.8, 154.5 (C-2 and C-5), and 195.6 (CHO). Tubes 0-24 were combined and taken to dryness. On column chromatography on Si gel, frustulosinol was eluted with EtOAc–petrol (1:1) and after crystallization from the same solvent mixture had mp 87–9°. The yield of these metabolites varied from batch to batch. In the best runs the yields were *ca* 3 mg/l. of culture liquid for frustulosin and *ca* 30 mg/l. for frustulosinol.

Acetylation of frustulosinol. Frustulosinol (0.2 g) was acetylated by refluxing with acetic anhydride (5 ml) and sodium acetate (0.5 g) for 1 hr. The acetate, crystallized from $CHCl_3$, had mp 76–8°, λ_{max}^{EtOH} 290 (*sh*), 273 (4.28) 260 (*sh*), 210 (4.4) nm; ν_{max} 1764, 1739, 1247, 1176 cm^{-1}. The PMR spectrum showed signals at 1.96 for the methyl protons, 2.02, 2.28 and 2.32 (3H each) for the acetate protons, 5.20 for the benzylic methylene, 5.32 and 5.38 for the olefinic protons, and 7.07 for the aromatic protons. The coupling pattern of the methyl and methylene protons of the side chain was the same as in the parent compound.

Hexahydrofrustulosinol. Frustulosinol (100 mg) was hydrogenated at room temperature and pressure in 20 ml ethyl acetate containing Pd/C (10%, 150 mg). Hydrogen uptake (3 mol) was complete in 10 min. The hexahydro derivative, crystallized from $CHCl_3$, had mp 136–8°, MS 210 (M^+), 192 ($M-H_2O$, base peak). Hexahydrofrustulosinol on refluxing with acetic anhydride and sodium acetate gave a triacetate, mp 62–64°.

Oxidation of frustulosinol. Frustulosinol (100 mg) was mixed with silver carbonate–Celite [7] (50 mg) in 10 ml C_6H_6. After 5

† This culture (No. 57048) was received from Dr. Ross W. Davidson, U.S.D.A., in December 1943.

min the mixture was centrifuged to remove inorganic matter, solvent was removed *in vacuo* and the residue crystallized from EtOAc–petrol (1 :2) to give the quinone (**5**) mp 62–64° (sublimes at 58°).

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Phyto—585/T.578—J.—Short Report

ISOLATION OF SOLAVETIVONE FROM *NICOTIANA TABACUM*

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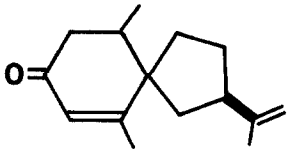
Key Word Index—*Nicotiana tabacum*; Solanaceae; Burley tobacco; vetispirane derivative; solavetivone

Preparative GLC of the neutral oil from 370 kg of air-cured American Burley tobacco leaves [1] afforded 10 mg colourless mobile oil (**1**) $[\alpha]_D^{23} - 92.8°$ (EtOH; *c* 0.15). The MS spectrum showed significant peaks at *m/e* 218(M^+, 48%), 137(58), 133(64), 108(100), 93(86), 79(72), 68(70), 67(70) and 41(74). By the high resolution MS, the formula of (**1**) was estimated as $C_{15}H_{22}O$ (found: 218.1676, calcd.: 218.1670). The IR spectrum showed an α,β-unsaturated carbonyl group at 1669 cm^{-1} and a terminal methylene group at 3090, 1650 and 893 cm^{-1}. No hydroxyl group was observed. The NMR spectrum (100 MHz, $CDCl_3$) showed a secondary methyl group (δ0.98, *d*, *J*7.0 Hz, 3H), a methyl group attached to a β-position of α,β-unsaturated carbonyl group (δ1.93, *d*, *J*1.2 Hz, 3H), an isopropenyl group (δ1.75, *br s*, 3H and δ4.68, *br s*, 2H), a methylene group adjacent to a carbonyl group (δ2.55, AB part of an ABX system) and an olefinic methine (δ5.62, *br s*, 1H). The UV spectrum had λ_{max}^{EtOH} 241.5 nm (ε14000) which was consistent with an enone system. These spectroscopic data of (**1**) were identical with those of solavetivone, 6,10-dimethyl-2-(1-methylethenyl)-spiro[4.5]dec-6-en-8-one, which has been isolated as a major stress metabolite produced by infected potato tubers [2]. This compound was also present in the leaves of *N. tabacum* cv Matsukawa, one of representative Japanese domestic tobacco, and Phillipine cigar tobacco (Manila leaves). This is the first report of the naturally existence of a vetispirane derivative in *N. tabacum*.



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