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BIOGENESIS AND REVISED STRUCTURE OF ROSELLISIN; STRUCTURE OF ROSELLISIN ALDEHYDE*

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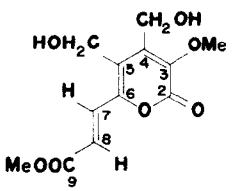
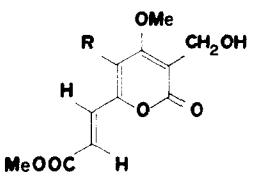
Key Word Index—*Hypomyces rosellus*; Hypocreaceae; structure determination; ^{13}C acetate incorporation; rosellisin; rosellisin aldehyde.

Abstract—On the basis of new data from biogenetic studies on rosellisin, the positions of the 3-OMe and the 4-CH_2OH have been interchanged, resulting in a revised structure. A new metabolite has been identified as rosellisin aldehyde.

In a preliminary communication [1] the structure **1** was proposed for the antibiotic fungal metabolite, rosellisin. The UV, IR, PMR and MS of rosellisin, its acetate and its dihydro derivative indicated two possible structures (**1** and **2**). However, the ^{13}C NMR spectrum strongly favored structure **1**: chemical shifts for the ring olefinic carbons were different from those typical for 4-oxy-α-pyrones. [2–4] and the chemical shift of the OMe, 63.2, was higher than would be expected for a methoxyl group in the 4-position [2, 3].



(1)



(2) R ≡ CH_2OH

(3) R ≡ CHO

Recent biogenetic studies involving incorporation of ^{13}C acetates have led to a reassignment of the ^{13}C chemical shifts. When rosellisin was labelled by incorporation of either ^{13}C-1 or ^{13}C-2 acetate, a set of four peaks in its ^{13}C NMR spectrum were enhanced: in the first instance, those at 125.1, 153.3, 163.8 and 167.7; in the second, those at 113.9, 118.7, 130.5 and 166.2. The previous assignments of these peaks were not compatible with alternate labelling. Accordingly, they have been reassigned as shown in Fig. 1. The observed chemical shift of the 4-OMe carbon, in the new assignment, may can be explained only as due to an abnormal influence of the hydroxyl groups.

Another basis for the original assignment of a 3-methoxyl group in rosellisin was the effect of copper acetylacetonate on the PMR spectrum; the two sharp singlets for the hydroxymethylenes collapsed to broad peaks and little or no broadening of the other peaks occurred. This was attributed to a bidentate complex formation. However, it can be explained on a different basis, which is compatible with structure **2**. Due to the planar nature of the carbon skeleton of the pyrone ring, monodentate complex formation at both the hydroxyls also would result in little broadening of the other peaks.

The biogenetic experiments establish that rosellisin is an acetogenin and not an isoprenoid like nectriapyrone [2], another α-pyrone isolated from a pyrenomycete. The only carbons which were labelled were the alternate ones of the C-2 to C-9 chain. The branch carbons of the hydroxymethylenes presumably originate from the "C1 pool".

A second compound, rosellisin aldehyde (**3**) was isolated in very small amounts from culture liquids of *Hypomyces rosellus*. This compound differs from rosellisin only in having an aldehyde rather than a hydroxymethylene group on C-5. Rosellisin aldehyde, isolated in semi-crystalline form, melted over an extended range, from 50–65°. However, it showed a single spot on TLC, in several solvents. It had MW 268 (CI-MS); λ_{max}^{EtOH} 348, 247 and 221 nm; ν_{max} 3450, 1724, 1700, 1639, 1597 and 1553 cm^{-1}; NMR signals at 2.8 (broad, 1H, OH), 3.80 (*s*, 3H, COOMe), 4.2 (*s*, 3H, OMe), 4.61 (*s*, 2H, CH_2OH), an AB quartet around 6.96 and 8.10 (1H each, *J* 15.5 Hz, C-8 and C-7 protons) and 10.18 (*s*, 1H, CHO). The downfield shift of the signals for olefinic protons in rosellisin aldehyde as compared to the corresponding signals

* Part 6 of the series, "Metabolites of Pyrenomycetes"; for part 5, see Carey, S. T. and Nair, M. S. R. (1975) *Lloydia* **38,** 448.

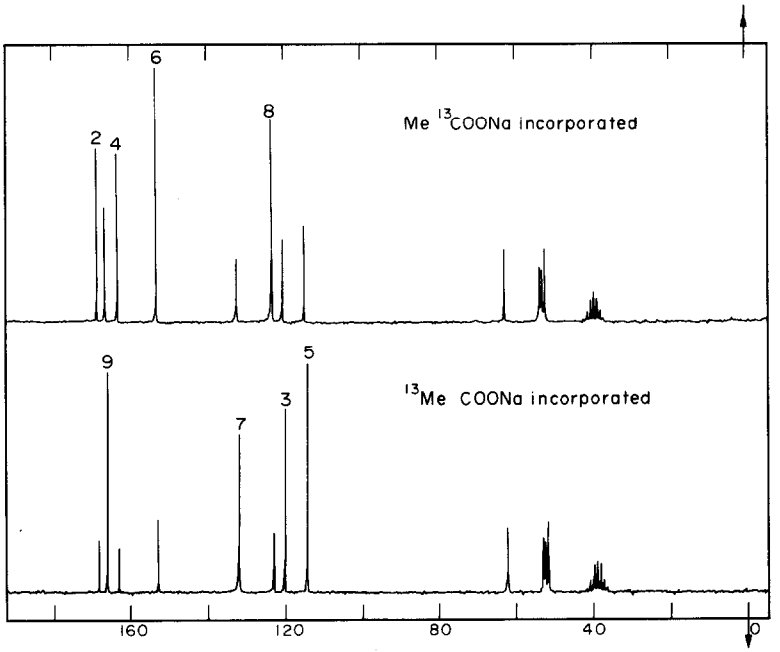


Fig. 1.

in the spectrum of rosellisin, shows that the aldehyde is at C-5, rather than at C-3. Similar downfield shifts of the olefinic proton signals occur in ortho substituted *trans*-cinnamic acid derivatives [5].

EXPERIMENTAL

General procedures. Mps are uncorrected; PMR spectra were taken in $CDCl_3$ with TMS as internal standard; ^{13}C NMR spectra were measured on a Jeol PS 100 spectrometer, with TMS as internal standard. Mass spectra were recorded by Morgan Shaffer Corp., Montreal, Canada, and microanalysis was carried out by Dr. Pascher, Bonn, West Germany.

Isolation of rosellisin and rosellisin aldehyde. Hypomyces rosellus was grown in still culture in a dextrose-yeast medium in Fernbach flasks in the dark at 25°. The culture was harvested after 21 days. In experiments in which ^{13}C acetates were incorporated, 1 g of 90% enriched acetate was added to 4 l. of the culture medium on the 11th day after inoculation. The culture liquid was extracted with EtOAc and the extract was taken to dryness *in vacuo*. On chromatography on a Si gel column (50 times w/w) rosellisin aldehyde was eluted by EtOAc and rosellisin by EtOAc–MeOH(2:1). The yield of rosellisin aldehyde was *ca* 2 mg/l. and that of rosellisin *ca* 75 mg/l. of culture liquid. Rosellisin, mp 110–112° (EtOAc), $C_{12}H_{14}O_7$ (elemental analysis) has MW 270 (MS); λ_{max}^{EtOH} 333 (4.10) and 228 nm (4.34); ν_{max} 3430, 1720 *sh*, 1703, 1669, 1600 and 1550 cm^{-1}; MS peaks at 270 (M^+, 40%) 239 (M–OMe, 45%), 211 (M–COOMe, 40%), 193 (211–H_2O, 80%) and 165 (193–CO, 100%), PMR signals at 2.85 (*s*, 2H exchange with D_2O, 2OH). 3.8 (*s*, 3H, COOMe), 4.03 (*s*, 3H, OMe), 4.53 and 4.56 (2*s*, 2H each, 2 CH_2OH) and an AB quartet centered at 6.62 and 7.67 (*J* 15.5 Hz, for C-8 and C-7) protons); ^{13}C NMR signals in $CDCl_3$ at 52.2 (COOMe), 54.6 and 55.6 (2 CH_2OH), 63.2 (OMe), 113.9 (C-5), 118.7 (C-3), 125.1 (C-8), 130.5 (C-7), 153.3 (C-6), 163.8 (C-4), 166.2 (COOMe) and 167.7 (C-2); in DMSO-D_6 some peaks are shifted: 120.3 (C-5), 123.0 (C-8) and 132.1 (C-7). The *diacetate* had mp 135–6° (EtOH), λ_{max}^{EtOH} 333 and 229 nm, ν_{max} 1750–1710 (broad) 1656, 1618 and 1567 cm^{-1} and PMR signals at 2.12 (*s*, 6H, acetates), 3.85 (*s*, 3H, COOMe), 4.1 (*s*, 3H, OMe), 5.04 and 5.08 (two singlets, 2H each $2CH_2 \cdot OAc$) 6.8 and 7.6 (2d, 1H each, *J* 15.5 Hz, olefinic protons).

Dihydrorosellisin. Rosellisin (100 mg) was hydrogenated at room temperature in presence of Pd/C (5%, 25 mg) for ten min. The main product was the 7, 8-dihydro derivative which was separated from minor impurities by PLC on a Si gel plate using EtOAc. Dihydrorosellisin had mp 101–2° (EtOAc); λ_{max}^{EtOH} 291 (3.87) and 206 nm (4.25); ν_{max} 3500, 1736, 1709, 1647 and 1563 cm^{-1} and PMR signals, 2.8 (*s*, superimposed on a broad peak 2.7–2.9, 4H, for 2 OH and 2 C-8 protons), 3.0–3.3 (broad peak, 2H, C-7 protons), 3.65 (*s*, 3H COOMe), 4.1 (*s*, 3H, OMe), 4.50 and 4.53 (2*s*, 2H each, 2 CH_2OH).

Antibiotic activity. Both rosellisin and rosellisin aldehyde were active against *Staphylococcus aureus* in serial dilution tests at a concentration of 30 ppm.

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