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Utilization of ^{13}C-^{13}C Coupling in Structural and Biosynthetic Studies. III. Ochrephilone - A New Fungal Metabolite[1]

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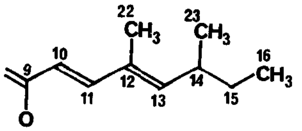
During biosynthetic studies with ^{13}C on metabolites[2] of *Penicillium multicolor* NRRL 2060, we isolated a new metabolite in addition to sclerotiorin[3] and rotiorin[4]. The spectral data suggested a similarity of the metabolite to the azaphilones, but unlike other azaphilones it failed to react characteristically with ammonia. The metabolite I, $C_{23}H_{26}O_5$ (M^+:m/e 382) mp. 135-140°(dec.), $[\alpha]_D^{25}$ +410° (c 0.44, ethanol), λ_{max}^{EtOH} 251 nm(ε=17500), 315(11900), 335(18300), 352(22900), 375(26200), 393(30500), 415(22000) and 442(9600), is a yellow pigment, which we named ochrephilone, and it gave a mono 2,4-dinitrophenylhydrazone derivative, $C_{29}H_{30}O_8N_4$ (M^+: m/e 562) mp. 232-233°C(dec.).

The presence of a γ-lactone, saturated ketone and conjugated ketone was indicated by the IR (ν_{max}^{nujol} 1773, 1730 and 1630-1620 cm^{-1}, respectively). The proton nmr spectrum of I indicated the presence of the side chain structure that is common to sclerotiorin and rotiorin.



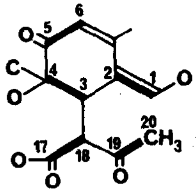
δ(in $CDCl_3$) 0.86(H_{16}, 3H, t, J=6.5Hz), 1.05(H_{23}, 3H, d, J=6.4Hz), ~1.35(H_{15}, 2H, m), 1.81(H_{22}, 3H, d, J=1.1Hz), ~2.45(H_{14}, 1H, m), 5.65(H_{13}, 1H, broad d, J=9.5Hz), 5.95 (H_{10}, 1H, d, J=15.5Hz) and 6.96(H_{11}, 1H, d, J=15.5Hz).

Signals in the pmr spectrum other than those from the side chain at [1.58(H_{21}, 3H, s), 2.44(H_{20} 3H, s), 5.40(H_6, 1H, d, J=0.7Hz), 6.15(H_8, 1H, s) and 7.40(H_1, 1H, broad s)] are also similar to those of rotiorin except for a characteristic singlet of two protons at δ 3.82. This two proton singlet appeared as an AB-quartet (δ 3.85, 1H, J=12.0Hz and 4.18, 1H, J=12.0Hz) in d_6-

acetone solution. These two protons with coincident chemical shifts, were established to be located on two adjacent methines from the cmr spectral data of I, the two doublets at δc 42.9 and 57.4 in the off-resonance decoupled spectrum (in $CDCl_3$) collapsed to two singlets on selective irradiation of the protons at δ 3.82.

Based on the structural similarity between I and rotiorin, whose polyketide origin has been established;[5] it appeared that I was also derived from a polyketide and several tentative structures in accord with the spectral data could be considered.

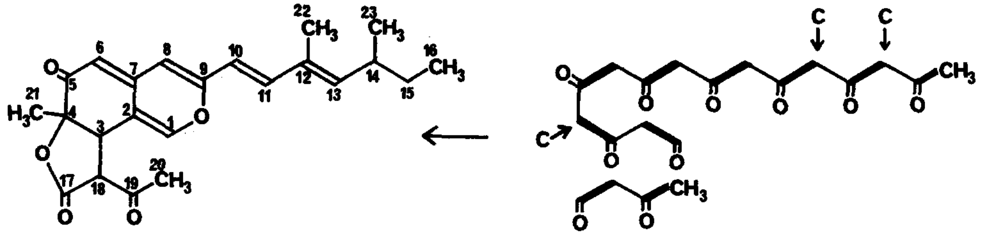
We turned to the use of the double labeling method[6,7] with $^{13}CH_3{}^{13}COONa$ (90% enriched) to obtain definitive structural information on I as well as biosynthetic information by ^{13}C-^{13}C coupling in the labeled metabolite. From this study, the following partial structure was established from the cmr spectrum of I (Fig. 1a). C_3 and C_{18} are the two methines described above.



δc from TMS (in $CDCl_3$) C_1(147.0), C_2(114.0), C_3(42.9), C_4 (82.9), C_5(191.0), C_6(106.1), C_{17}(168.7), C_{18}(57.4), C_{19} (200.1), and C_{20}(30.2). The ^{13}C-^{13}C coupling constants[8] observed are as follows: $J_{1,2}$=78Hz, $J_{3,4}$=34Hz, $J_{5,6}$=61Hz, $J_{17,18}$=48Hz, and $J_{19,20}$=44Hz. An oxygen substituent at C_1 is indicated by the large ^{13}C-^{13}C coupling constant between C_1 and C_2, and by the chemical shift of H_1(δ 7.40). The relationship between C_4 and C_5, and C_{18} and C_{19} was established by observing the following 1,3-couplings[9] with I labeled with $^{13}CH_3COONa$ (Fig. 1b): $J_{4,6}$=14Hz;z and $J_{18,20}$=12Hz. Proton decoupling experiments proved the relationship between H_1 and H_3 (J_{H_1,H_3}< 0.5Hz), and H_1 and H_6 (J_{H_1,H_6}=1.0Hz). Thus, C_4 and C_{17} should be linked through oxygen to form a γ-lactone.

The structure of the side chain is also supported by ^{13}C-^{13}C coupling observed with double labeled I: C_9(157.1), C_{10}(116.2), C_{11}(141.5), C_{12}(131.9), C_{13}(147.3), C_{14}(35.0), C_{15}(30.1), C_{16} (11.9), C_{22}(20.2), and C_{23}(12.3). $J_{9,10}$=69Hz, $J_{11,12}$=56Hz, $J_{13,14}$=44Hz, and $J_{15,16}$=36Hz. The magnitude of the coupling constant between C_{11} and C_{12} agrees very well with that reported for 1,3-butadiene.[10] The chemical shift of C_9 together with the large coupling constant between C_9 and C_{10} as compared with C_{11} and C_{12} clearly indicates the attachment of an electronegative substituent[8] at C_9. The remaining carbons [=C_7-C_8H=, C_7(144.7) and C_8(107.6), $J_{7,8}$=54Hz)] should be incorporated into the total structure of I as shown on the next page. The carbon linked to C_4 is therefore assigned to the C_{21} methyl (δc 23.2).

The structure is further confirmed by the ^{13}C-^{13}C coupling constants observed with I obtained from a labeling experiment with a 1:1 mixture[6] of $^{13}CH_3COONa$ and $CH_3{}^{13}COONa$ (both 90% enriched): $J_{2,3}$=42Hz, $J_{4,5}$=42Hz, $J_{6,7}$=72Hz**, $J_{8,9}$=72Hz**, $J_{10,11}$=70Hz, $J_{12,13}$=70Hz, $J_{14,15}$=35Hz, and $J_{18,19}$=40Hz.



In the cmr spectrum of I labeled with $^{13}CH_3COONa$, signals of C_2, C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, and C_{20} appeared with increased intensity (approximately 2.5 times) as compared with the unlabeled compound. Thus, I is biosynthesized from 10 molecules of acetic acid without C-C bond cleavage as shown above with the introduction of three C_1-units, presumably from formate or methionine as in the case of sclerotiorin[11] and rotiorin[5].

I is structurally related to rubrorotiorin[12] and appears to be derived by reduction of the corresponding dechloro compound. Another metabolite which we consider to be dechlororotiorin from the proton nmr spectral data, was detected in the mycelium of the fungus.

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** Due to the overlapping of the signals, these values were obtained by the distance between the central peak and the satellite peak.

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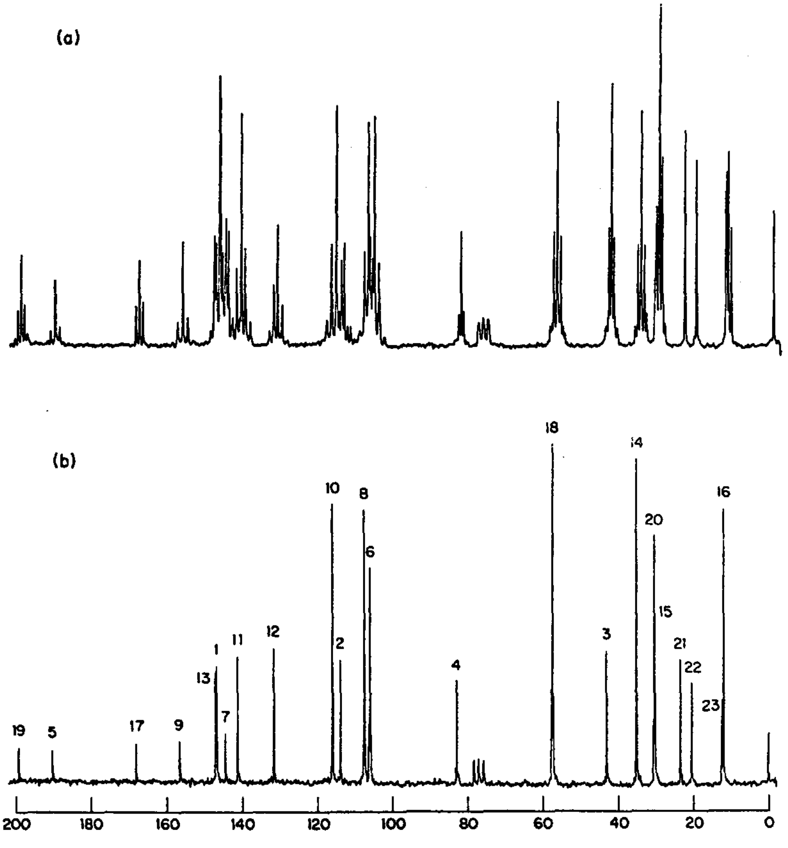

Fig. 1. Proton noise-decoupled cmr spectra of ochrephilone from (a) $^{13}CH_3{}^{13}COONa$ and (b) $^{13}CH_3COONa$.