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THE MARTICINS: EVIDENCE FOR A MIXED ORIGIN FROM THE POLYKETIDE AND TRICARBOXYLIC ACID PATHWAYS BY [2-$^{13}C_1$] AND [1,2-$^{13}C_2$]-ACETATE INCORPORATION EXPERIMENTS

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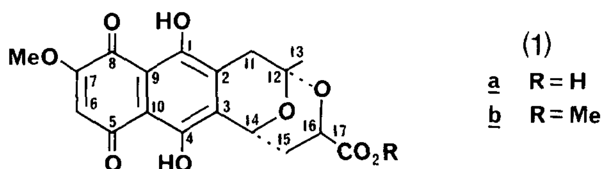
Abstract: ^{13}Cmr spectra of the methyl ester of marticin, biosynthetically enriched with ^{13}C from [2-$^{13}C_1$] and [1,2-$^{13}C_2$]acetate, indicate a mixed biogenesis from a heptaketide and a Krebs cycle acid.

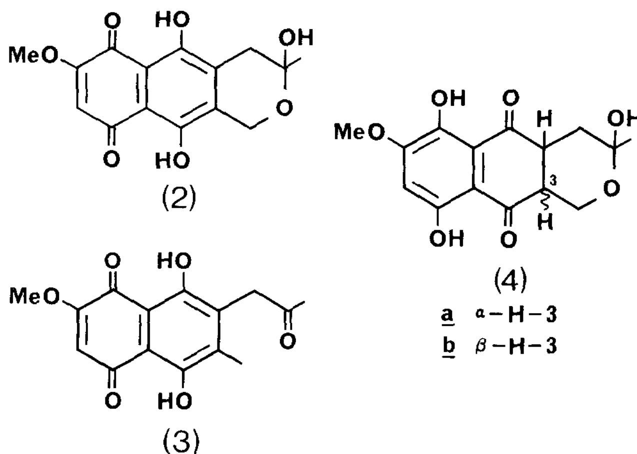
Marticin and its less stable stereomer isomarticin are phytotoxic metabolites of Fusarium spp. of the group Martiella. Their isolation and biological properties are well documented[1-3] but an account of the studies leading to the assignment of structure **1a** is available only in thesis form.[4] On the basis of time-sequence studies,[1] Kern and Naef-Roth suggested that the compounds were the precursors of their congeners fusarubin (**2**) and javanicin (**3**), with the implication that the marticins are true nornonaketides. This hypothesis, however, conflicts with the rule that the "uncyclized residues from the methyl ends of polyketide chains are never shorter than residues from the carboxyl end of the chain",[5] and with the recent demonstration that in F. solani f. sp. cucurbitae, fusarubin arises nonenzymatically from the dihydrofusarubins (**4a** and **4b**) which are regarded as primary metabolites of the fungus.[6,7] An alternative origin of the marticins, by the addition of a C_3 unit to a heptaketide moiety, was therefore discussed as more probable.[8]

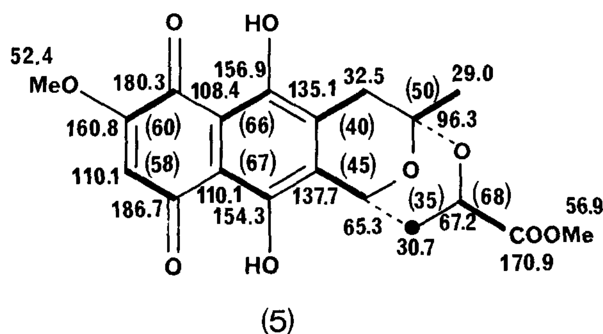
To verify this proposal, shake cultures of F. martii App. et Wr. var. pisi F.R. Jones on Raulin medium (15 x 80 mL) were supplemented with sodium [1,2-$^{13}C_2$]acetate (0.1 M; 4 mL/flask) just after the color began to turn orange (3 days) and again after another day (deep

orange turning red). The $NaHCO_3$ soluble portion of the EtOAc extract obtained from the filtered culture medium on day 5, containing isomarticin together with smaller amounts of marticin and 2 (tlc comparison with authentic samples), afforded marticin methyl ester (1b) on brief heating in methanolic HCl and recrystallization ($MeOH/CHCl_3$) of the precipitated product: 10 mg, m.p. 236-238°C; $\delta_H(CDCl_3)$: 12.9 (1H, s, OH), 12.5 (1H, s, OH), 6.22 (1H, s, H-6), 5.57 (1H, dd (J = 2, 5 Hz), H-14), 4.40 (1H, dd (J ~ 3, 12.5 Hz), H-16), 3.95 (3H, s, CO_2Me), 3.74 (3H, s, OMe), 3.11 (1H, d (J = 20 Hz), H-11), 3.01 (1H, d (J = 20 Hz), H-11), 2.42 (1H, ddd (J = 5, 12.5, 13 Hz), H-15α), 2.02 (1H, ddd (J = 2, 3, 13 Hz), H-15β) and 1.70 (3H, s, Me). These data are in accord with those reported by Pfiffner[4] for the monomethyl ether of 1b, and also agree well with the ^{1}Hmr data reported recently by Tatum and Baker[9] for marticin with a reassignment of multiplets at 5.57 and 4.40 ppm to H-14 and H-16, respectively (our numbering).

The ^{13}Cmr spectrum of 1b established that all carbons, with the exception of the methoxyls, were enriched and each of the signals corresponding to those for the natural abundance material were accompanied by ^{13}C satellites, demonstrating the pairwise incorporation of carbons in intact acetate units for C-1 through C-14 as shown in 5. Furthermore, most of the patterns exhibited satellites of the satellites undoubtedly owing to the relatively high probability of incorporation of labelled acetate units into adjacent sites during the limited period of active uptake[7] of exogenous acetate; such coupling between acetate units has been termed "induced" coupling.[10] The relative intensities of the satellites to the central signals indicated ^{13}C enrichment of ca. 2% for the carbons of the fusarubin moiety. For C-15, -16 and -17, the ^{13}C enrichment was significantly lower (ca. 1%). The relative intensity of the satellites with J = 68 Hz indicated that C-16 and -17 were incorporated as an intact acetate unit but the C-16 pattern also contained less intense satellites arising from coupling with C-15 confirming its enrichment. Although C-16 and -17 arise from an intact acetate unit, this unit cannot be part of an initial nonaketide chain since the latter would be required to go through an improbable rearrangement with the extrusion of a carbon atom. It follows that 1a arises from a fusarubin (or precursor) skeleton to which is added a C_3 unit derived, most probably, from succinate, oxalacetate or other metabolite of the tricarboxylic acid (Krebs) cycle into which the labelled acetate can enter with facility and in the required manner.[11]







As a further test of this conclusion, cultures of the fungus were supplemented with [2-$^{13}C_1$]-acetate. The isolated marticin methyl ester (1b) exhibited a ^{13}Cmr spectrum in which the signals for the alternate carbons of the fusarubin moiety were enhanced as were those for C-15 and -16 which correspond to C-2 and -3 of succinate, both derived from C-2 of acetate after one or more passages through the Krebs cycle.[11] Furthermore the prominent C-15 and -16 signals were accompanied by ^{13}C satellites, J = 36 Hz, indicating a high level of enrichment in the Krebs cycle precursor.

On the basis of these observations we conclude that the marticins are produced by the

addition of a C_3 Krebs cycle precursor to a heptaketide which is probably related to the intermediate leading to fusarubin and congeners.

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References

1. H. Kern and S. Naef-Roth, Phytopathol. Z. 53, 45 (1965).
2. H. Kern, S. Naef-Roth and H. Item, Phytopathol. Z. 67, 1 (1970).
3. H. Kern, Ann. Phytopathol. 10, 327 (1978).
4. A. Pfiffner, Isolierung und Konstitutionermittlung von Marticin und Isomarticin, zwei neuen Welketoxinen aus Fusarium Martii. Dissertation No. 3361 (E. Hardegger, supervisor), ETH, Zurich, 1963.
5. W.B. Turner, "Fungal Metabolites", Academic Press, London and New York, 1971, p. 198.
6. I. Kurobane, L.C. Vining, A.G. McInnes and D.G. Smith, Can. J. Chem. 56, 1593 (1978); I. Kurobane, L.C. Vining, A.G. McInnes and N.C. Gerber, J. Antibiot. 33, 1376 (1980);
7. I. Kurobane, L.C. Vining, A.G. McInnes and J.A. Walter, Can. J. Chem. 59, 1380 (1980).
8. A. Stoessl in "Toxins in Plant Disease" (R.D. Durbin, ed.), Academic Press, New York, 1981, p. 146.
9. J.H. Tatum and R.A. Baker, Phytochemistry 22, 543 (1983).
10. A.P.W. Bradshaw, J.R. Hanson and M. Siverns, J.C.S. Chem. Comm. 819 (1977).
11. H. Beevers, M.L. Stiller and V.S. Butt in "Plant Physiology" (F.C. Steward, ed.), Academic Press, London and New York, 1966, p. 119.

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