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Tenellin and Bassianin, Metabolites of *Beauveria* Species. Structure Elucidation with ^{15}N- and Doubly ^{13}C-Enriched Compounds using ^{13}C Nuclear Magnetic Resonance Spectroscopy

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Summary ^{13}C n.m.r. spectroscopy and biosynthetic labelling with [^{15}N]nitrate and [1,2-^{13}C]acetate proved to be valuable adjuncts to established chemical and spectroscopic methods in elucidating the structures of tenellin and bassianin as 3-acyl derivatives of 1,4-dihydroxy-5-*p*-hydroxyphenyl)-2(1*H*)-pyridone.

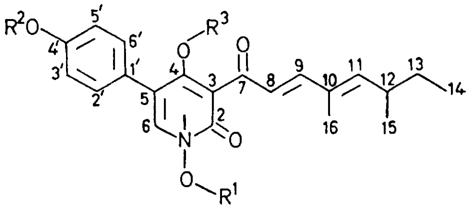
A PREVIOUS publication[1] contains preliminary u.v., i.r., and mass spectral data on two related pigments tenellin (I) ($C_{21}H_{23}NO_5$), and bassianin ($C_{23}H_{25}NO_5$), as well as conditions for their production and isolation from the insect pathogenic fungi *Beauveria tenella* (Delacroix) Siem., and *Beauveria bassiana* (Bals.) Vuill. We now report their structures.

Methylation of (I) with Ag_2O–MeI progressively methylates the hydroxamic acid [(II); m.p. 194°], phenol [(III); m.p. 96—98°], and enol [(IV); wax] hydroxy-groups. High resolution mass spectral studies, including precursor ion experiments, showed a major fragmentation pathway *via* initial loss of oxygen in (I), whereas in contrast fragmentation of (II), (III), and (IV) involved initial loss of CH_2O. This provides strong evidence for a cyclic hydroxamic acid unit[2] in (I).

Compound (I), $[\alpha]_D^{24}$ $-$ 44·0° (1% in acetone) with alkaline peroxide gave *p*-hydroxybenzoic acid; in refluxing KOH by retro-aldol cleavage it gave the 3-acetyl derivative (V), m.p. 230—231° ($C_{13}H_{11}NO_5$, *m/e* 261·0635), and two aldehydes, both racemic mixtures, characterized as the 2,4-dinitrophenylhydrazones of 2-methylbutanal, m.p.[3] 128·5° and 2,4-dimethylhex-2-enal, m.p.[4] 160—161°.

The ^{1}H n.m.r. signals [$(CD_3)_2SO$; 100 MHz] of (I) could be assigned as follows: δ 17·0br [s, 1H, –C(OH)=C(C=O)–], 11·5br (s, 1H, >NOH), 9·5br (s, 1H, ArOH; this and previous OH's temperature dependent, exchanged with D_2O), 8·10 (s, 1H, 6-H), 7·78 [AB, 2H, 8- and 9-H, $\Delta\nu_{AB}$ 50·4, J_{AB} 15·3 Hz, 9-H long range coupled <0·5 Hz to 11-H (double resonance)], 7·07 (AA'BB', 4H, $\Delta\nu_{AB}$ 49·6 Hz, J_{AB} 8·6 Hz, 1,4-subst. Ar), 5·92br [d, 1H, 11-H, $J_{11,12}$ 9·6 Hz, coupled <0·3 Hz to vinylic Me (double resonance)], 2·50 (m, 1H, 12-H, Bu^s- >CH-), 1·88br (s, 3H, 16-H, vinylic Me), 1·38 (m, 2H, 13-H, Bu^s- >CH_2), 1·01 (d, 3H, 15-H, Bu^s-Me, $J_{12,15}$ 6·3 Hz), and 0·85br (t, 3H, 14-H, remaining Bu^s-Me). The only significant differences in the spectra of derivatives were: (II), δ 11·5 (OH) replaced by δ 3·98 (>NOMe); (III), δ 11·5 and 9·5 (OH) replaced by δ 4·02 (>NOMe) and 3·79 (ArOMe); (V), all signals for H-8 replaced by acetyl Me at δ 2·70. Structural, stereochemical, and conformational features of (I) are thus retained in (II) and (III); (V) differs only in the contraction of an acyl to an acetyl substituent. A nuclear Overhauser enhancement (NOE) study[5] [irradiated proton(s) in brackets] of (II) gave: 9-H {11-H} 22%; 8-H {11-H} 0%; 9-H {allylic Me} 0%; 8-H {allylic Me} 20%; 6-H {>NOMe} 8%; 6-H {2′- and 6′-H} 26%; 3′- and 5′-H {2′- and 6′-H} 10%.

The preceding evidence requires that (I), (II), and (III) possess 4,6-dimethylocta-*trans,trans*-2,4-dienoyl and HOC_6H_4C units, with 6-H close to the chemically equivalent aromatic hydrogens 2′-H and 6′-H, as well as to the *N*-methoxy-group in (II) and (III), and hence the *N*-hydroxy-group in (I).



(I) $R^1 = R^2 = R^3 = H$
(II) $R^1 = Me; R^2 = R^3 = H$
(III) $R^1 = R^2 = Me; R^3 = H$
(IV) $R^1 = R^2 = R^3 = Me$

Pulse Fourier transform (8K; data accuracy ±0·6 Hz) ^{13}C n.m.r. spectral data [$(CD_3)_2SO$; internal reference Me_4Si, 5 kHz sweep width, 25·16 MHz] were obtained from proton-noise decoupled (p.n.d.) and high resolution spectra, and assignments were aided by off-resonance and single ^1H frequency decoupling experiments.[6,7] Assignments for (I) were: δ 193·8 (C-7), 173·0 (C-4), 157·5 (C-2), 156·9 (C-4′), 150·8 (C-11, $^1J_{CH}$ 154·4 Hz), 149·7 (C-9, $^1J_{CH}$ 155·9 Hz), 140·0 (C-6, $^1J_{CH}$ 183·6 Hz), 132·6 (C-10), 130·2 (C-2′, -6′, $^1J_{CH}$ 160·0 Hz), 123·1 (C-8, $^1J_{CH}$ 167·5 Hz, 122·8 (C-1′), 115·0 (C-3′, -5′, $^1J_{CH}$ 160·0 Hz), 110·9 (C-5), 105·9 (C-3), 34·6 (C-12, $^1J_{CH}$ 125·4 Hz), 29·4 (C-13, $^1J_{CH}$ 126·0 Hz), 19·8 (C-15, $^1J_{CH}$ 125·8 Hz), 12·3 (C-16, $^1J_{CH}$ 126·2 Hz), 11·7 (C-14, $^1J_{CH}$ 125·2 Hz).

Addition of 90% enriched [1,2-^{13}C] acetate to cultures of *B. bassiana* yielded (I) containing five discrete two-carbon units each doubly labelled with ^{13}C. The presence of ^{13}C–^{13}C spin–spin coupling in the p.n.d. spectrum proved the existence of the following pairs of directly bonded carbons: C-2, C-3 ($^1J_{CC}$ 75·3 Hz); C-7, C-8 ($^1J_{CC}$ 55·9 Hz); C-9, C-10 ($^1J_{CC}$ 53·6 Hz); C-11, C-12 ($^1J_{CC}$ 43·2 Hz); C-13, C-14 ($^1J_{CC}$ 34·4 Hz).

In addition the p.n.d. spectrum of (I) 95% enriched with ^{15}N (obtained from cultures with $K^{15}NO_3$ as the only nitrogen source) established that only C-6 [$^1J(^{15}NC)$ 15·0 Hz], C-2 [$^1J(^{15}NC)$ 11·0 Hz], and C-3 [^2J (^{15}NC) 9·2 Hz] were spin–spin coupled to ^{15}N, and only H-6 [$^2J(^{15}NH)$ 1·0 Hz] was similarly coupled to nitrogen in the corresponding ^1H n.m.r. spectrum. These data confirm that the nitrogen in (I) is geminal to 6-H and C-3, and directly bonded to C-6 and C-2;[8] the latter observation provides further evidence for a hydroxamic acid group.

The combined results therefore require that tenellin possesses a 1-hydroxy-2-pyridone ring substituted as shown in structure (I). Our NOE study places H-6 and the *p*-hydroxyphenyl substituent on adjacent carbons. C-4, C-3, and C-7 must constitute the enol form of a β-diketone system in order to account for their chemical shift,[7a] and the presence of the strongly hydrogen bonded hydroxy-group observed in the ^1H n.m.r. spectrum; the chemical shift of C-5 is consistent with it being deshielded by the aryl group and shielded by β-nitrogen and hydroxy substituents. Tenellin is therefore 3-(4,6-dimethyl-*trans-trans*-octa-2,4-dienoyl)-1,4-dihydroxy-5-(*p*-hydroxyphenyl)-2(1*H*)-pyridone.

Parallel studies on bassianin gave a structure differing only in that the C-3 acyl substituent is extended by an additional conjugated *trans*-substituted –CH=CH– unit positioned between C-7 and C-8 of the structure (I).

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