Glyantrypine, a Novel Anthranilic Acid-containing Metabolite of *Aspergillus clavatus*

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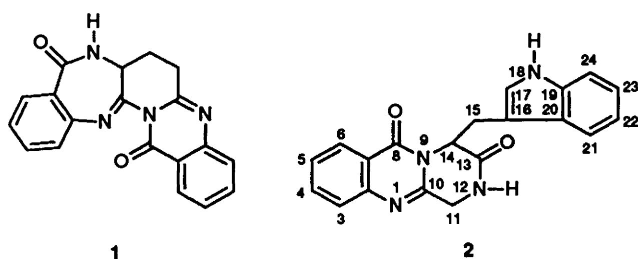
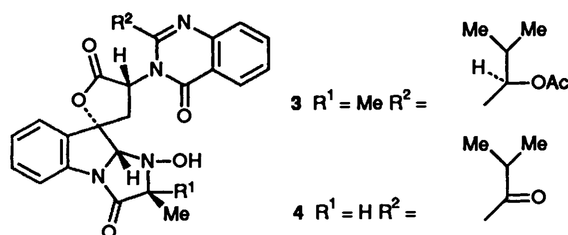
The structure of a novel indole alkaloid, glyantrypine **2**, a main secondary metabolite of *Aspergillus clavatus* has been elucidated by biosynthetic evidence, mass spectrometry and ^{1}H and ^{13}C NMR techniques. The compound extends the variety of metabolites biosynthesised from anthranilic acid and tryptophan without forming a benzodiazepine.

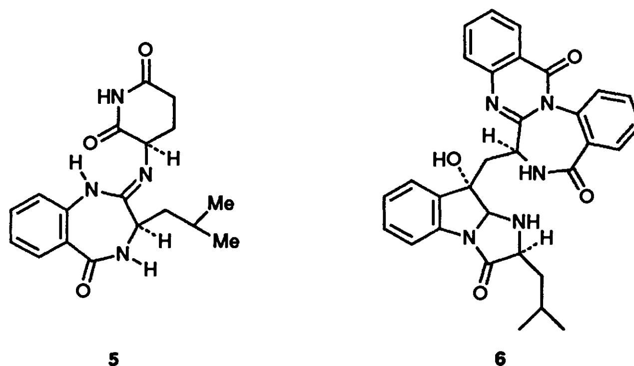
Benzodiazepine fungal metabolites are formed by the condensation of anthranilic acid and an α-amino acid. Therefore benzodiazepines, such as auranthine **1** produced by *Penicillium*

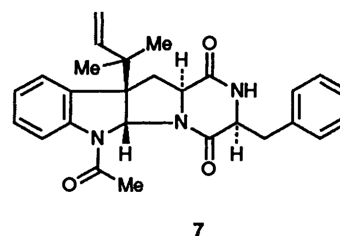




aurantiogriseum, may be detected using a [*carboxyl*-^{14}C]-anthranilic acid probe.[1] Further screening of fungi for similar compounds has revealed a prominent anthranilic acid-containing metabolite which is designated glyantrypine **2** on account of its biosynthetic precursors. However, **2** is not a benzodiazepine. Glyantrypine is produced by a Yugoslavian isolate of *Aspergillus clavatus*, isolates of which species otherwise produce the non-benzodiazepine metabolites tryptoquivaline **3** and tryptoquivalone **4** which also contain anthranilic acid and tryptophan residues.

Biosynthetic experiments, reported in detail elsewhere,[2] showed significant incorporation of radiolabel into **2** from [*carboxyl*-^{14}C]anthranilic acid (0.05%), [*methylene*-^{14}C]-tryptophan (0.07%) and [1-^{14}C]glycine (0.14%). The presence of an indole in **2** was evident from the electron impact mass spectrum showing a prominent fragment ion at *m/z* 130. An ion at *m/z* 174 was interpreted as containing anthranilic acid and glycine moieties. Other fragments at *m/z* 215, 186 and 160 were consistent with the deduction that **2** was composed of anthranilic acid, tryptophan and glycine residues, condensed with the overall loss of three water molecules but without the formation of a seven-membered benzodiazepine ring.





The ^{1}H NMR spectrum of **2** in [$^{2}H_6$]dimethyl sulfoxide([$^{2}H_6$]DMSO) revealed nine aromatic protons, a broad NH doublet coupled to one proton of a methylene system, (δ 3.79, dd), and another three spin system (δ 5.27, t; δ 3.32, dd; δ 3.42, q) which is essentially the same as that given by the α and β protons of tryptophan. There was an indolic NH signal at δ 10.93. The proton–proton correlation spectrum confirmed the presence of the CH–CH_2 and NH–CH_2 three spin systems. These observations are consistent with the structure **2**, which was initially suggested by the mass spectrometry and biosynthesis experiments, but do not by themselves lead to a complete elucidation of the structure. Connectivity between aromatic protons was established by use of the two-dimensional COSY spectrum and by analogy with resonances for a novel benzodiazepine **5** (biosynthesised by a particular isolate of *P. aurantiogriseum* from Yugoslavia and composed of anthranilic acid, leucine and glutamine residues),[3] tryptophan[4] and other model compounds,[4] which assisted assignment of protons. Molecular modelling studies employing MOPAC (version 5.2) with the PM3 Hamiltonian afforded an optimised geometry where dihedral angles of 15 and 104° between the protons at C-11 and the adjacent NH proton of the amide were observed; this is consistent with the observed couplings.

^{13}C NMR spectroscopy of **2** in [$^{2}H_6$]DMSO (Table 1) used

Table 1 ^1H and ^{13}C NMR data for glyantrypine **2** in [$^2H_6$]DMSO

Position	δ_H	δ_C	J^a/Hz
2	—	147.00	—
3	7.56 dd	126.31	3,4 7.5; 3,5 1.7
4	7.83 dt	137.46	4,3 7.5; 4,5 8.0; 4,6 2.0
5	7.55 dt	126.62	5,6 8.5; 5,4 8.0; 5,3 1.7
6	8.21 dd	126.76	6,5 8.5; 6,4 2.0
7	—	119.88	—
8	—	167.63	—
10	—	149.31	—
11′	(3.04 d	43.79	(11.11′ 17.0
11	3.79 dd)		11′,11 17.0; 11′,12 4.5)
12	8.32 d	—	12,11′ 4.5
13	—	159.96	—
14	5.27 t	56.47	14,15 5.4; 14,15′ 4.5
15	(3.32 dd	26.52	(15,15′ 9.3; 15,14 5.4
15′	3.42 q)		15′,15 9.3; 15′,14 4.5)
16	—	124.41	—
17	6.86 d	107.78	17,18 2.4
18	10.93 br s	—	(br)
19	—	136.02	—
20	—	127.16	—
21	7.31 dd	118.63	21,22 7.8; 21,23 1.3
22	7.00	121.22	22,21 7.8; 22,23 7.2; 22,24 1.0
23	6.77 dt	117.75	23,24 7.8; 23,22 7.2; 23,21 1.3
24	7.26 dd	111.43	24,23 7.8; 24,22 1.0

^{a} Measured from a resolution enhanced spectrum.

DEPT experiments to enable assignment of the proton multiplicities. The spectra revealed eight quaternary carbons, eleven CH groups and two CH_2 groups. Comparison of this data with the resonances obtained in [$^2H_6$]DMSO for **1**,[5] **5**,[3] tryptophan and other model compounds,[4] asperlicin **6**[6] and a tryptophan-phenylalanine diketopiperazine **7**[3] (described as fructigenine A[7]) identified structural fragments which supported the structure **2** and led to the assignments reported in Table 1.

Model compounds such as **1** were particularly useful in assigning the carbonyl resonances at C-8 and C-13 and the imine resonance at C-10 within six-membered rings. Also notable was the similarity of the aromatic resonances assigned to the indole moiety in **2** with those of tryptophan, rather that with those of the more constrained indole system in **6**.[6]

Experimental

Fermentation Production of Glyantrypine.—500 cm^3 Erlenmeyer flasks containing 100 cm^3 medium were incubated at 27 °C on a rotary shaker. Seed stage cultures in Czapek Dox–yeast extract (0.5%) broth (CDYE medium) were inoculated with spores of a Yugoslavian isolate of *A. clavatus*[8] (IMI 349510) and grown for 24 h. A 5% v/v transfer of seed stage culture to CDYE medium supplemented with $CaCl_2$ (2%) initiated the production stage. Chloroform extracts of broth analysed by silica gel TLC in chloroform–acetone (1:1) showed that, typically, **2** was first evident on day 3 and reached maximum yield by day 10 (2.1 mg, 100 cm^{-3} medium). The filtrate from a 40 flask batch was extracted twice with two half volumes of chloroform. Combined extracts were evaporated to dryness and the residue, dissolved in chloroform–acetone (1:1), processed by flash chromatography[9] through silica gel 60 (Merck; 230–400 mesh) in a column (5 × 30 cm). Elution with chloroform–acetone (1:1) gave 50 cm^3 fractions in which **2** was mainly in fractions 5–8. Further purification of **2** was performed by HPLC through a Spherisorb ODS 1 reversed phase column (0.5 × 25 cm) with methanol–water (55:45) at 0.5 cm^3 min^{-1} and detection at 219 nm (retention time 25 min), yield 10 mg. Analytical HPLC used methanol–water (77:23) at 1 cm^3 min^{-1} (retention time 5.3 min).

Glyantrypine was isolated as a white amorphous solid with λ_{max}(in methanol) 224 and 270 nm (Found M$^+$, 344.1279. $C_{20}H_{16}N_2O_4$ requires *M*, 344.1273. Major fragment ions at *m/z* 215, 186, 174, 160 and 130).

NMR measurements were made under standard conditions at 500 MHz (^1H) or 125.8 Hz (^{13}C) on a Bruker AM-500. Mass spectrometry used a VG-7070 mass spectrometer.

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