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QUERCETIN 3,7,3′-TRISULPHATE FROM *FLAVERIA BIDENTIS*

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Key Word Index—*Flaveria bidentis*; Compositae; quercetin 3,7,3′-trisulphate.

Abstract—From leaves of *Flaveria bidentis* a new quercetin trisulphate was isolated and characterized as quercetin 3,7,3′-trisulphate by means of spectroscopic (UV, ^{1}H NMR, ^{13}C NMR) and chemical methods.

In previous papers on the constituents of leaves of *Flaveria bidentis* (L) O.K. [1–3] we have reported the isolation and structure elucidation of several quercetin sulphates. Other flavonoid sulphates have been obtained from members of the Dilleniaceae [4]. The present communication describes the isolation and characterization of a new quercetin sulphate from *F. bidentis*.

RESULTS AND DISCUSSION

Extraction of dried and ground leaves of *F. bidentis*, as previously reported [3], yielded a crude extract that was purified by chromatography on a Sephadex G 10 column eluted with water. This procedure afforded a product (**1**) which was pure as judged by PC (Whatman 3 MM, water) and electrophoretic analysis. Acid hydrolysis of **1** gave quercetin (mp and UV data) and sulphate. Its UV spectrum indicated the presence of a free hydroxyl group at C-4′, confirmed by comparison with the UV spectra of quercetin 3,7,4′-trisulphate [3] and of the permethylated product [5]. The ^{1}H NMR spectrum presented five aromatic protons in a pattern similar to that observed for other quercetin sulphates. The ^{13}C NMR spectrum (Table 1) was assigned by comparison with that of quercetin [6, 7] and taking into account the expected upfield displacement (*ca* 5–6 ppm) for aromatic carbon atoms having an *O*-sulphate group and the downfield shift (*ca* 3–4 ppm) for the α-carbons, all in accordance with tabulated ^{13}C NMR data [8]. Accordingly, hydroxyl groups at positions 3, 7 and 3′ appeared to be esterified by sulphate groups. This result is in agreement with that obtained by UV analysis characterizing **1** as quercetin 3,7,3′-trisulphate which to the best of our knowledge has not been previously described.

Table 1. ^{13}C NMR spectral data of quercetin 3,7,3′-trisulphate (25.2 MHz, DMSO-d_6)

Carbon	δ (ppm)	Carbon	δ (ppm)
2	154.9	10	106.0
3	132.5	1′	123.0
4	177.6	2′	120.8
5	159.0*	3′	140.1
6	102.2	4′	152.2
7	159.9*	5′	116.6
8	97.5	6′	126.8
9	156.5		

*Values may be interchanged.

EXPERIMENTAL

Plant material and isolation procedure. These have been previously reported [1–3].

Quercetin 3,7,3′-*trisulphate* (**1**). Golden yellow crystals. UV $\lambda_{max}^{MeOH-H_2O}$ nm: 230 sh, 243 sh, 268, 322 sh, 342; + NaOMe: 243, 267 sh, 272, 300 sh, 391; + $AlCl_3$: 234 sh, 256 sh, 276, 300 sh, 340, 387; + $AlCl_3$ + HCl: 234 sh, 256 sh, 270, 300 sh, 340, 385; + NaOAc: 266, 397. ^{1}H NMR (100 MHz, DMSO-d_6): δ 6.58 (1H, *d*, *J* = 2 Hz, H-6), 6.90 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.92 (1H, *d*, *J* = 2 Hz, H-8), 7.95 (1H, *d*, *J* = 2 Hz, H-2′), 8.03 (1H, *dd*, *J* = 8.5 and 2 Hz, H-6′). Found: S, 14.46%. $C_{15}H_7O_{16}S_3K_3$ requires: S, 14.63%.

Quercetin 5,4′-*dimethyl*-3,7,3′-*trisulphate* (**2**). Compound **1** (20 mg) was treated with an ethereal soln of CH_2N_2 in a sealed tube with occasional shaking for 7 days. The reaction mixture was purified by PPC affording a product which did not react with $FeCl_3$. UV $\lambda_{max}^{MeOH-H_2O}$ nm: 258, 322; the spectrum did not shift after addition of the reagents indicated above.

Acid hydrolysis of quercetin 5,4′-*dimethyl*-3,7,3′-*trisulphate* (**2**). Compound **2** was hydrolysed with 0.1 N HCl in a sealed tube at 100° for 1 hr. The UV of the resulting product showed λ_{max}^{MeOH} nm: 253, 268 sh, 300 sh, 363; + NaOMe: 268, 318 sh, 395; + $AlCl_3$: 262, 286 sh, 423; + $AlCl_3$ + HCl: 262, 286 sh, 421; + NaOAc: 270, 320 sh, 397, indicating a quercetin 5,4′-dimethyl ether [5].

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NIGRIFORTINE, A DIKETOPIPERAZINE METABOLITE OF *PENICILLIUM NIGRICANS*

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Key Word Index—*Penicillium nigricans*; nigrifortine; diketopiperazine; biosynthesis.

Abstract—The structure of a novel diketopiperazine metabolite, named nigrifortine, isolated from cultures of *Penicillium nigricans* is deduced from its ^{1}H NMR, mass and UV spectra together with biosynthetic reasoning.

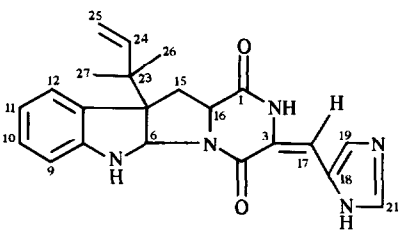
INTRODUCTION

In recent years an increasing number of simple and substituted diketopiperazine alkaloids, derived from two amino acids, have been isolated from fungi. Over 40 such substances are listed [1, 2] as fungal metabolites, the biosynthesis of which frequently involves one or more aromatic amino acid precursors. Although a strain of *Penicillium nigricans* has been shown to elaborate the simple symmetrical dimer L-phenylalanine anhydride [3], neither the equivalent dimer of tryptophan nor a derivative of it has yet been reported.

RESULTS AND DISCUSSION

In the course of studies on the biosynthesis of the indolic penitrem mycotoxins an isolate of *P. nigricans* in our laboratory [4] became the focus of attention since it could be induced by calcium chloride to sporulate in submerged fermentation and, concomitantly, produce penitrem mycotoxins together with the antibiotic griseofulvin [5]. Mycelial extracts, made from shaken flask cultures given [*benzene ring-U*-^{14}C]tryptophan during the phase in which penitrems are biosynthesized, were found also to contain a less polar substance which, from TLC autoradiography, was evidently derived from tryptophan. The yield of the compound, 6 mg from the mycelium grown submerged in 100 ml of the medium, was *ca* twice that of penitrems and thus it was at least a principal secondary metabolite produced under these conditions. The specific activity of the metabolite derived from [^{14}C]tryptophan was 4.27×10^4 dpm/mg, an incorporation of 4%. Cultures given [2-^{14}C]mevalonic acid also incorporated the radiolabel into the metabolites with similar efficiency (specific activity of metabolite, 3.64×10^4 dpm/mg).

Fast atom bombardment (FAB) and electron impact (EI) mass spectrometry gave the molecular formula $C_{32}H_{36}N_4O_2$ and fragmentation losses equivalent to two isoprenes. The loss of the first isoprene is analogous to the loss (69 mass units = C_5H_9) evident in the fragmentation of roquefortine (**1**) [6], a substituted indolic diketo-



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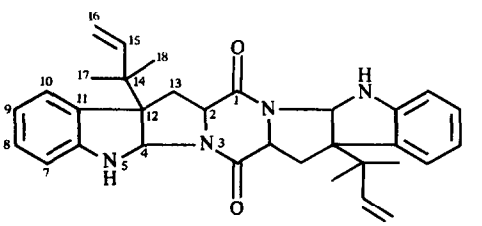

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