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12,13-DEOXYTRICHOVERRINS FROM *MYROTHECIUM VERRUCARIA*

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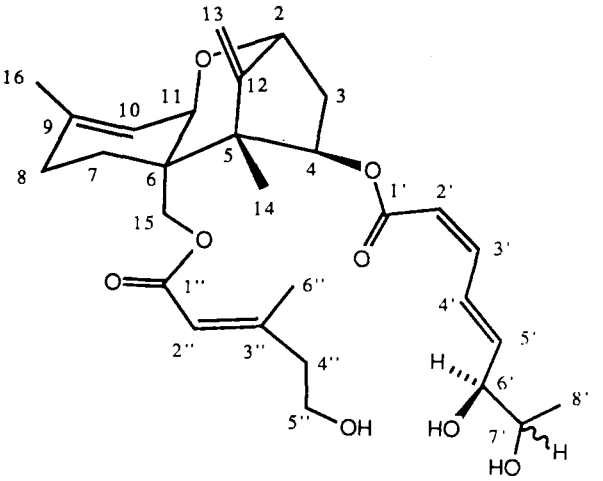
ABSTRACT.—The structures of two new diastereoisomeric 12,13-deoxytrichothecenes have been established by a combination of nmr and mass spectroscopy.

The trichothecenes constitute an important class of mycotoxins which exhibit a broad spectrum of biological activity (1). We have isolated a variety of macrocyclic trichothecenes (2) from cultures of *Myrothecium* (3), some of which we have used as starting materials for the preparation of chemically modified anticancer macrocyclic trichothecenes (4). During the course of the isolation of known verrucarins and roridins from a 340-liter fermentation of *Myrothecium verrucaria* CL-72, we isolated a set of new diastereoisomeric trichoverroids. Spectral data, particularly ^{1}H and ^{13}C nmr, made it clear that these compounds were closely related to the trichoverrins (5) but that they lacked the 12,13-epoxy functionality, i.e., they are 12,13-deoxytrichoverrins.

The deoxytrichoverrins A [**1**] and B [**2**] were present in the culture at low levels (< 1 mg/liter) and proved very difficult to separate from the larger amounts of trichoverrols A and B and trichoverrins A and B. The most salient features of the ^{1}H-nmr spectra of **1** and **2** are the lack of the AB signals from the 12,13-epoxy protons (~3 ppm) and the appearance of the C-13 geminal olefinic singlets at ca. 4.7 and 5.1 ppm. The stereochemistry at C-6′ and C-7′ can be assigned based on ^{13}C-nmr data for **1** and **2** and the ^{1}H-nmr data for their triacetates: **1** is 6′(*S*), 7′(*S*) (L-*threo*) and **2** is 6′(*S*), 7′(*R*) (D-*erythro*); the absolute stereochemistry at C-6′ and C-7′ is assumed to be the same as in the trichoverrols and trichoverrins (5). The signal for H-7′ in the triacetate of **1** appears at δ 5.2 as a five-line multiplet, with $J_{7',8'} \approx J_{6',7} = 6$ Hz; whereas in the triacetate of **2** this signal appears at δ 5.1 as an eight-line multiplet with $J_{7',8} = 6$ Hz and $J_{6',7'} = 4$ Hz. The carbon signals at C-6′ and C-8′ in **1** resonate about 1 ppm higher than do these carbons in **2**. These data are entirely consistent with those observed with similar *threo* and *erythro*



1 C-7′ = (*S*)
2 C-7′ = (*R*)

diastereomers of the trichoverroids (5) as well as with the macrocyclic trichothecenes (6).

The 12,13-deoxytrichothecenes have been reported previously as minor metabolites of *M. verrucaria* (7,8) and *Fusarium graminearum* (9). They also have been shown to be generated in vivo through metabolism of trichothecenes in a variety of mammalian systems (10,11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns hot-stage melting point apparatus and are uncorrected. Ir spectra were determined in $CHCl_3$ on a Perkin-Elmer Model 281 spectrometer. Nmr spectra were determined in $CDCl_3$ on an IBM SY-200 MHz spectrometer with TMS as an internal standard; ^{13}C-nmr peaks were assigned by using INEPT and by comparison of chemical shift data with those in the literature. Mass spectra were determined on a VG 7070E mass spectrometer in the fab mode [$CDCl_3$-glycerol (1:4) matrix]. Triacetates of **1** and **2** were prepared by dissolving 5-mg samples in 500 μl each of Ac_2O and pyridine. After 1 day, 5 ml of H_2O was added, and the mixture was extracted with 3 × 5 ml of Et_2O. The Et_2O extracts were combined, washed with 5% HCl, 5% $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated. Tlc analysis (20% EtOAc/hexane, SiO_2) showed only a single spot.

Fermentation procedure with *M. verrucaria* CL-72 was carried out in a manner similar to that described previously (5). A lower R_f fraction (4.2 g), whose polarity (as judged by tlc, 70% EtOAc/hexane, SiO_2) was just slightly lower than that of trichoverrols A and B (5) and slightly higher than that of trichoverrins A and B (5), was obtained following a series of cc. Rotary preparative tlc [2-mm plates, EtOAc/hexane (50-100% EtOAc), SiO_2] (Harrison Research Chromatotron Model 7942) followed by preparative hplc (10 mm × 250 mm, 5 μ amino Spherisorb column, Altex Model 332 hplc, 70% hexane/15% iPrOH/15%CH_2Cl_2, flow rate 4 ml/min) gave 380 mg of a mixture of **1** and **2**. Reversed-phase hplc (10 mm × 250 mm, 5 μ C8, Spherisorb column, 45% H_2O/55% MeOH, flow rate 3 ml/min) gave 12 mg of **1** and 63 mg of **2**.

12,13-DEOXYTRICHOVERRIN A [**1**].—Oil; uv (EtOH) λ max nm (log ε) 260 (4.12); ir ($CHCl_3$) ν max cm^{-1} 3500 (OH), 1705 (C=O), 1645 (C=C); hrms (fab) *m/z* $[M+H]^+$ 517.2833, calcd 517.2801 for $C_{29}H_{40}O_8+H$; ^{1}H nmr ($CDCl_3$) δ 0.95 (3H, s, H-14), 1.21 (3H, d, *J* = 6 Hz, H-8′), 1.65 (3H, s, H-16), 2.15 (3H, s, H-6″), 2.40 (2H, m, H-4″), 2.50 (1H, dd, *J* = 8, 15 Hz, H-3α), 4.06 (2H, AB, *J* = 12 Hz, H-15), 4.38 (1H, d, *J* = 5 Hz, H-2), 4.70 and 5.13 (1H each, s, H-13), 5.42 (1H, d, *J* = 5 Hz, H-10), 5.51 (1H, d, *J* = 11 Hz, H-2′), 5.84 (1H, s, H-2″), 6.12 (1H, dd, *J* = 6, 16 Hz, H-5′), 6.22 (1H, dd, *J* = 4, 8 Hz, H-4), 6.63 (1H, dd, *J*'s = 11 Hz, H-3′), 7.60 (1H, dd, *J* = 11, 16 Hz, H-4′); ^{13}C nmr ($CDCl_3$) δ 10.8 (C-14), 18.8 (C-8′), 19.2 (C-6″), 21.3 (C-7), 23.2 (C-16), 27.9 (C-8), 37.9 (C-3), 42.3 (C-6), 43.5 (C-4″), 51.2 (C-5), 59.5 (C-5″), 64.2 (C-15), 66.4 (C-11), 70.4 (C-7′), 75.0 (C-4), 76.3 (C-6′), 78.8 (C-2), 105.2 (C-13), 117.0 (C-2″), 117.6 (C-2′), 118.8 (C-10), 127.4 (C-4′), 140.0 (C-9), 142.1 (C-5′), 144.3 (C-3′), 152.2 (C-12), 156.6 (C-3″), 166.0 (C-1′ and C-1″).

12,13-DEOXYTRICHOVERRIN B [**2**].—Mp 84–86° (hexane/Et_2O); uv (EtOH) λ max nm (log ε) 260 (4.12); ir ($CHCl_3$) ν max cm^{-1} 3500 (OH), 1705 (C=O), 1645 (C=C); hrms (fab) *m/z* $[M+H]^+$ 517.2790, calcd 517.2801 for $C_{29}H_{40}C_8+H$; ^{1}H nmr ($CDCl_3$) δ 0.95 (3H, s, H-14), 1.16 (3H, d, *J* = 6 Hz, H-8′), 1.64 (3H, s, H-16), 2.15 (3H, s, H-6″), 2.40 (2H, m, H-4″), 2.51 (1H, dd, *J* = 8, 15 Hz, H-3α), 4.05 (2H, AB, *J* = 12 Hz, H-15), 4.38 (1H, d, *J* = 5 Hz, H-2), 4.70 and 5.12 (1H each, s, H-13), 5.42 (1H, d, *J* = 5 Hz, H-10), 5.50 (1H, d, *J* = 11 Hz, H-2′), 5.84 (1H, s, H-2″), 6.11 (1H, dd, *J* = 6, 16 Hz, H-5′), 6.22 (1H, dd, *J* = 4, 8 Hz, H-4), 6.64 (1H, dd, *J*'s = 11 Hz, H-3′), 7.55 (1H, dd, *J* = 11, 16 Hz, H-4′); ^{13}C nmr ($CDCl_3$) δ 10.8 (C-14), 17.9 (C-8′), 19.2 (C-6″), 21.3 (C-7), 23.2 (C-16), 27.9 (C-9), 37.9 (C-3), 42.3 (C-6), 43.5 (C-4″), 51.2 (C-5), 59.5 (C-5″), 64.2 (C-15), 66.4 (C-11), 70.2 (C-7′), 75.0 (C-4), 75.3 (C-6′), 78.8 (C-2), 105.2 (C-13), 117.0 (C-2″), 117.8 (C-2′), 118.8 (C-10), 127.4 (C-4′), 140.0 (C-9), 141.2 (C-5′), 144.3 (C-3′), 152.2 (C-12), 156.6 (C-3″), 166.0 (C-1′ and C-1″).

ACKNOWLEDGMENTS

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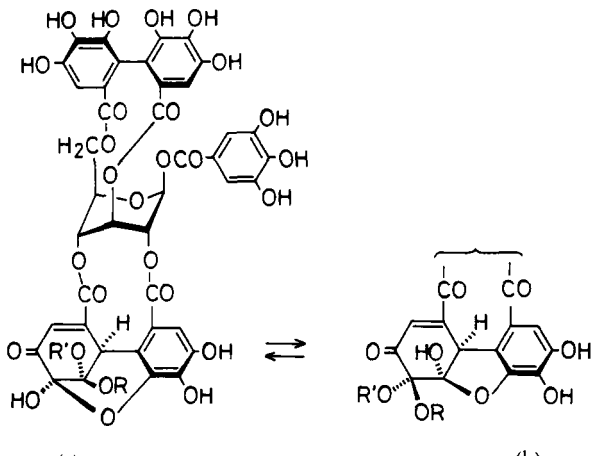
ERRATUM

The authors have requested the following correction for the paper entitled "New Methods of Analyzing Tannins," *J. Nat. Prod.*, **52**, 1 (1989).

The corrected structures **10** and **11** are as follows:



10 R=R′=H geraniin
11 R=H, R′=Me(or Et)

SCHEME 1.