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Isolation, Chemical Structure, Acute Toxicity, and Some Physicochemical Properties of Territrem B′ from *Aspergillus terreus*

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We have isolated a metabolite of territrem, designated territrem B′, from the chloroform extract of a rice culture of *Aspergillus terreus* 23-1 by using the same isolation procedure as that for territrems A, B, and C. The present isolation procedure gave about 10 mg of territrem B′ from 4 kg of rice culture per batch. Analysis of the high-resolution mass spectrum showed that the molecular composition of territrem B′ is $C_{29}H_{34}O_{10}$ (found, 542.2167; required, 542.200). Some results of physicochemical and acute tests are presented in this paper. Single-crystal X-ray diffractometry of territrem B′ indicated that the three-dimensional structure of territrem B′ has not changed significantly from that of territrem B except for the insertion of one oxygen atom into territrem B to make an additional pyron ring in the E ring. The tremorgenic activity of territrem B′ is greatly reduced as tested by intraperitoneal injection in mice.

A series of structurally related tremorgenic metabolites, designated territrems A, B, and C (TRA, TRB, and TRC, respectively), was isolated from the chloroform extract of a rice culture of *Aspergillus terreus* 23-1 (2, 4). Recently, we have succeeded in isolating another related metabolite from the condensed fraction I of the chloroform extract of the same rice culture. Since the R_f values of the compound in some thin-layer chromatography systems (Fig. 1) were between the R_f values of TRB and TRC, the compound was designated territrem B′ (TRB′). The procedures for culture, clean up on a silica gel column, silica gel column chromatography, and thin-layer chromatography were described by Ling et al. (3). Fraction I was concentrated to dryness by a rotation evaporator, redissolved in a minimal amount of chloroform, and precipitated with an excess amount of *n*-hexane. The dried precipitate, about 800 mg from one batch, containing TRA, TRB, and TRB′ was dissolved in a minimal amount of chloroform, applied to a column of Silica Gel 60, 4 cm (inner diameter) by 29 cm long, and eluted with benzene-ethyl acetate (1:1, vol/vol) at a flow rate of 1 ml/min. Each tube collected 20 ml of effluent, and the compounds in each tube were detected by thin-layer chromatography. The fractions containing TRB′ were pooled, dried by a rotation evaporator, and dissolved in chloroform-ethanol (1:1, vol/vol) for crystallization. The solvent for the final recrystallization was chloroform-benzene (5:1, vol/vol). This isolation procedure gave about 10 mg of TRB′, 81 mg of TRA, 662.4 mg of TRA plus TRB, and 214 mg of TRB from 4 kg of rice culture per batch.

The procedures for analysis of some physicochemical properties of TRB′ were followed as described previously (4). High-resolution mass spectra were analyzed with an AEI MS-30 spectrometer (Shrader Analytical Consulting Laboratories Inc., Detroit, Mich.). The melting point of TRB′ was 222 to 224°C (uncorrected). The optical rotation (α_D) ($CHCl_3$) was +102° (*c*:0.01 g/ml). The excitation and emission wavelengths of fluorescence spectra in methanol were 375 and 420 nm, respectively. The molar absorptivities of TRB′ at 331 and 219 nm in methanol were 19,753 and 33,598, respectively, and 18,815 at 335 nm in chloroform.

A comparison of the infrared absorption spectra of TRB′ and TRB shows that both have nearly the same functional groups, except that the peak corresponding to the ketone C=O stretch in TRB′ is not as intense as that in TRB, but the ester carbonyl C=O stretch in TRB′ is more intense than that in TRB. High-resolution mass spectral analysis of TRB has shown that its molecular composition is $C_{29}H_{34}O_{10}$ (found, 542.2167; required, 542.2001). The difference in molecular composition between TRB′ and TRB ($C_{29}H_{34}O_9$) is therefore 16 (O). In a comparison of proton magnetic

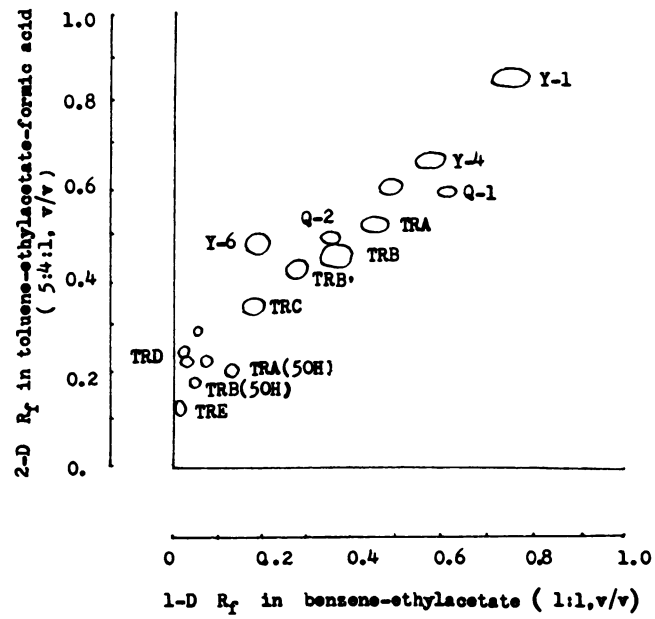

FIG. 1. Two-dimensional thin-layer chromatogram of chloroform extracts from the moldy rice infected with *A. terreus* 23-1. Spots were viewed under either long-wave or short-wave UV light. There are three groups of spots: blue fluorescent compounds involving territrems, yellow-green compounds designated as Y-series under long-wave UV light, and fluorescence-quenching spots designated as Q-series under short-wave UV light.

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resonance (PMR) signals in $CDCl_3$ from $(CH_4)_4Si$ of TRB′ and TRB, it is found that the PMR signals at δ3.90 (s, 9H) and δ6.97 (s, 2H) in TRB′ correspond well to those signals at δ3.90 (s, 9H) assigned as three methoxy protons and signals at δ6.95 (s, 2H) assigned as two protons of aryl-2H in the aryl moiety of TRB (4). These PMR spectral data also correlated well with the mass spectral results. Since the base peak of the benzoyl cation, *m/e* 195 ($C_{10}H_9O_4^+$), of TRB was also present in the mass spectra of TRB′ as the base peak, it is concluded that the structural difference between TRB′ and TRB is in their non aromatic moiety. Twelve protons of four isolated methyl groups (C-23 to C-26 in Fig. 2A) at δ1.23 (s, 3H), δ1.31 (s, 3H), δ1.49 (s, 3H), and δ1.55 (s, 3H) in TRB were replaced by nine protons at δ1.075 (s, 3H), δ1.273 (s, 3H), and δ1.327 (s, 3H) in TRB′. The signals at δ5.769 to 5.874 (d, J = 10 Hz, 1H) and δ6.259 (d, J = 10 Hz, 1H) in TRB assigned to HC-20 and HC-21 were missing in TRB′. However, the signals at δ6.32 (s, 1H) assigned to HC-9 and at δ2.83 (d, J = 17 Hz, 1H) and δ3.45 (d, J = 17 Hz, 1H) assigned to two protons at C-12 in TRB were also present in TRB′. D_2O exchangeable signals at δ4.020 (s, 1H) and δ5.987 (s, 1H) of TRB were assigned to two hydroxy groups located at C-17 and C-13. In TRB′, with the addition of D_2O, the signal at δ4.718 (s, 1H) shifted to δ4.694 and the signal at δ4.091 (s, 1H) disappeared.

TRB′ crystallized into an orthorhombic configuration (space group $p2_12_12_1$) with the following cell dimensions: a = 0.8840(3), b = 1.2484(2), c = 2.4432(4) nm; Z = 4, D_x = 1.34, D_m = 1.33 g cm^{-3}. The intensity was measured with M_0K radiation on a Nonius CAD4 diffractometer by θ/2θ scan techniques. The scan range was calculated by 0.8 + 0.7tanθ + 0.8 in 2θ. A total of 4,409 reflections were measured with

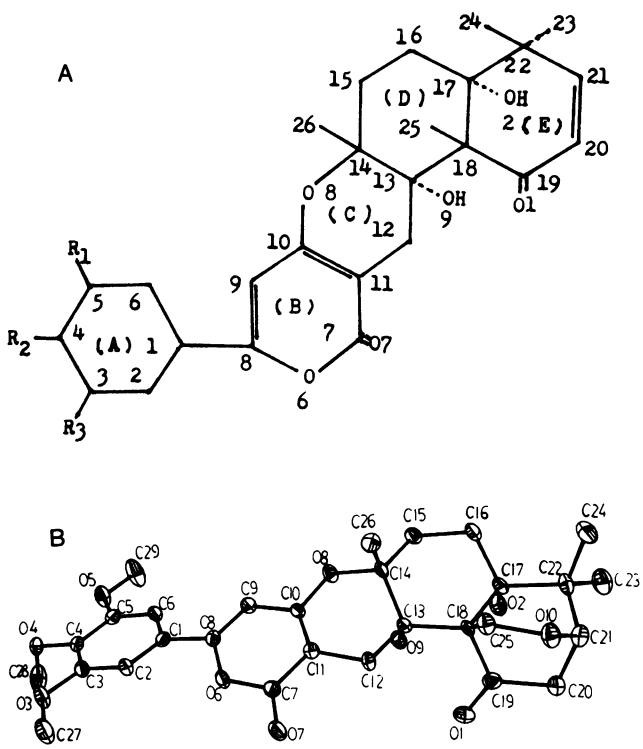

FIG. 2. (A) Structure of territrems: TRA, $R_1 + R_2 = OCH_2O$, $R_3 = OCH_3$; TRB, $R_1 = R_2 = R_3 = OCH_3$; TRC, $R_1 = R_3 = OCH_3$, $R_2 = OH$. (B) Stereoscopic view of TRB′.

TABLE 1. Bond lengths

Bond[a]	Length (nm)
C-1 to C-2	0.1381(8)
C-1 to C-6	0.1395(8)
C-1 to C-8	0.1467(7)
C-2 to C-3	0.1391(8)
C-3 to O-4	0.1391(8)
C-3 to O-3	0.1364(7)
C-4 to C-5	0.1383(8)
C-4 to O-4	0.1365(6)
C-5 to C-6	0.1396(8)
C-5 to O-5	0.1364(7)
C-7 to C-11	0.1423(8)
C-7 to O-6	0.1390(7)
C-7 to O-7	0.1209(7)
C-8 to C-9	0.1331(8)
C-8 to O-6	0.1387(6)
C-9 to C-10	0.1431(7)
C-10 to C-11	0.1343(7)
C-10 to O-8	0.1354(6)
C-11 to C-12	0.1513(7)
C-12 to C-13	0.1523(7)
C-13 to C-14	0.1552(7)
C-13 to C-18	0.1567(7)
C-13 to O-9	0.1451(6)
C-14 to C-15	0.1507(8)
C-14 to C-26	0.1525(8)
C-14 to O-8	0.1483(6)
C-15 to C-16	0.1542(8)
C-16 to C-17	0.1543(8)
C-17 to C-18	0.1580(7)
C-17 to C-22	0.1582(7)
C-17 to O-2	0.1444(7)
C-18 to C-19	0.1542(7)
C-18 to C-25	0.1536(8)
C-19 to C-20	0.1506(8)
C-19 to O-1	0.1214(7)
C-20 to C-21	0.1518(9)
C-21 to C-22	0.1518(9)
C-21 to O-10	0.1439(7)
C-22 to C-23	0.1548(9)
C-22 to C-24	0.1563(8)
C-25 to O-10	0.1438(7)
C-27 to O-3	0.1394(8)
C-28 to O-4	0.1436(8)
C-29 to O-5	0.1426(8)

[a] Designations correspond to those shown in Fig. 2B.

a variable scan speed of 20/3 to 20/17 degrees/min, of which 2,171 were considered to be observed with $1 \geq 2\sigma$ (1) and were used on the subsequent least-square refinements. Three reflections were monitored every 2 h throughout the measurement. The intensity variations were less than 2% for 5 days. The crystal structure of TRB′ was solved by a direct method involving the use of the program MULTAN with 282 top *e* values and 50 lowest *e* values. All the non-hydrogen atoms of rings A, B, C, and D appeared in the *e* map. Subsequent Fourier synthesis gave the rest of the non-hydrogen atoms. The least-square refinements coverged to the agreement factors of $R = 0.056$ and $R_w = 0.043$, with a total of 353 variables. Some of the hydrogen atoms were found in the difference Fourier map, and the rest were calculated by assuming tetrahedral or trigonal symmetry around the carbon atoms. The molecular structure of TRB′ (Fig. 2B) is very similar to that of TRB (1). They can be described as isomorphous with similar unit cell dimensions and packings. The only difference is on the E ring, in which the methyl group on C-18 in TRB becomes a —CH_2O— group bonded to C-21 of the E ring to form an extra six-membered ring. Therefore, the E ring becomes a bicyclic structure. The double bond between C-20 and C-21 in TRB (0.131 nm) becomes a single bond (0.1518 nm). This structure solves the puzzle of where the extra oxygen atom is and is consistent with all the spectroscopic observations stated above. The molecular structure and the thermal ellipsoids are shown in Fig. 2B. The bond lengths are given in Table 1.

The procedure for the acute toxicity test and the test animals used were the same as those in Ling et al. (4). Neither tremor nor other pathological symptoms were observed in mice after intraperitoneal injection of TRB′ at a dose of 1 mg per mouse. However, higher doses were not tried. The modification of the A ring such as in TRA and TRC did not appreciably affect their tremorgenic activity (2, 4). However, an insertion of one oxygen atom into TRB to make an additional pyron ring in the E ring greatly reduced its tremorgenic activity. However, from single-crystal X-ray diffraction, the three-dimensional structure of TRB′ is not much changed from that of TRB. Further study of the structure-activity relationship of territrems is in progress in our laboratory.

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