Four New Mycotoxins of *Aspergillus clavatus* Related to Tryptoquivaline

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The hydroxylamine nortryptoquivaline (**2**) and the three secondary amines deoxytryptoquivaline (**3**), deoxynortryptoquivaline (**4**), and deoxynortryptoquivalone (**8**) were found to be toxic metabolites of *Aspergillus clavatus*. They were accompanied by the two previously described tremor producing agents tryptoquivaline (**1**) and nortryptoquivalone (**7**). The only weakly basic secondary amines **3**, **4**, and **8** were oxidized to the corresponding hydroxylamines **1**, **2**, and **7** with *m*-chloroperbenzoic acid.

A strain of the fungus *Aspergillus clavatus* (NRRL-5890) collected from mold-damaged rice produced the two highly toxic, tremor inducing metabolites tryptoquivaline (**1**) and nortryptoquivalone (**7**).[3] The *p*-bromophenylurethane of a transformation product of tryptoquivaline was utilized in the structure determination by x-ray crystallography while spectral data left little doubt that nortryptoquivalone[4] has structure **7**, but this conclusion remains to be confirmed by chemical correlation.

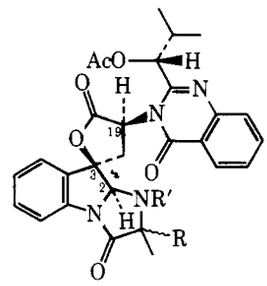
More recently we had occasion to examine another strain of *Aspergillus clavatus* (strain MIT-M-18) for the presence of mycotoxins. Its identity was established by the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The fungus was one of several collected from mold-infested rice found in a Thai household where a child died of an unidentified toxicosis.[5] Solid substrate, agitated fermentation on pearled barley[6] gave optimum yields of secondary metabolites and individual components were isolated by high-pressure liquid and thin layer chromatography. The least and most polar fractions contained the known fungal metabolites xanthocillin-X dimethyl ether (**9**)[7] and kotanin (**10**),[8] respectively. From the intermediate fractions nortryptoquivalone (**7**) and tryptoquivaline (**1**) could be isolated in crystalline form. These two metabolites were now found to exhibit very similar circular dichroism spectra (see Experimental Section) tentatively suggesting identical *relative* configurations at C_2, C_3, and C_{19}.[9]

The least polar of the new metabolites, mp 256–258 °C, further characterized by its acetate **5**, had the composition $C_{28}H_{28}N_4O_7$. Its ultraviolet absorption spectrum was indistinguishable from that of tryptoquivaline (**1**) and all infrared absorptions associated with functional groups were identical in the two spectra. The proton magnetic resonance spectrum of the new metabolite was identical with that of tryptoquivaline (**1**) except that signals associated with the geminal dimethyl group in the latter were replaced by those of a secondary methyl function. The first of the new metabolites thus is nortryptoquivaline (**2**) and its circular dichroism spectrum indicates the relative stereochemistry shown.

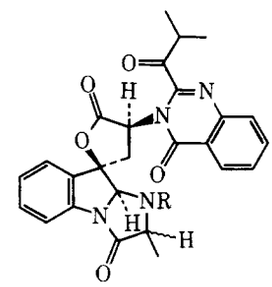
A second new metabolite, mp 150–152 °C, was found to have the composition $C_{29}H_{30}N_4O_6$. It thus differs from tryptoquivaline (**1**) by the absence of an oxygen atom. The suggestion that it might be deoxytryptoquivaline (**3**) was strengthened by a negative triphenyltetrazolium chloride test for hydroxylamines[12] and confirmed by oxidation to tryptoquivaline (**1**) with *m*-chloroperbenzoic acid. Deoxytryptoquivaline (**3**) is only weakly basic and was not extracted from organic solvents by 1 N aqueous hydrochloric acid.

The third of the new mycotoxins, mp 192–193 °C, with the composition $C_{26}H_{24}N_4O_5$ was deoxynortryptoquivalone (**8**) oxidized to nortryptoquivalone (**7**) by *m*-chloroperbenzoic acid.

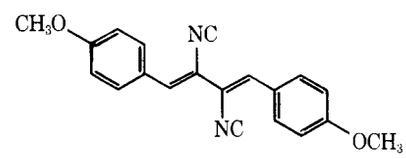
A negative color test for hydroxylamines suggested that the last metabolite, mp 158–160 °C, $C_{28}H_{28}N_4O_6$, is also a secondary amine and its oxidation to nortryptoquivaline (**2**) with peracid established it to be deoxynortryptoquivaline (**4**). Direct comparison[10] with a substance called FTD (later shown to be norisotryptoquivaline[11]) elaborated by *Aspergillus fumigatus* proved their nonidentity. All four of the new metabolites are toxic and full toxicological data will be reported by Professor Gerald Wogan elsewhere. None have activity against the mycotoxin assay organism, *Bacillus megaterium*.[6] On the other hand, the xanthocillin-X dimethyl ether showed antibacterial activity when assay disks were dipped into solutions containing 125 μg/ml or higher.



1, R = CH_3; R′ = OH
2, R = H; R′ = OH
3, R = CH_3; R′ = H
4, R = H; R′ = H
5, R = H; R′ = OAc
6, R = H; R′ = Ac



7, R = OH
8, R = H



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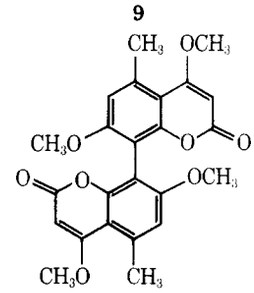

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Experimental Section

Melting points were determined on a Kofler hot-stage microscope and are corrected. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. Ultraviolet (UV) spectra were determined on a Cary 14 recording spectrophotometer. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 567 grating spectrophotometer. Nuclear magnetic resonance (NMR) spectra were measured on a Hitachi Perkin-Elmer R-22 90-MHz instrument and are given in parts per million (δ) downfield from an internal tetramethylsilane standard;

the abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. High-resolution mass spectra (HRMS) were determined at 70 eV on a CEC-110B (Du Pont) instrument. Circular dichroism (CD) spectra were measured on a Cary 60 spectrophotometer. High-pressure liquid chromatography (HPLC) was performed using a Waters Associates liquid chromatograph with a 7 ft × 0.375 in. o.d. Porasil A (0.037–0.075 mm particle size) column, and about 20 mg per separation.

Production and Isolation of the Secondary Fungal Metabolites. *A. clavatus* (strain MIT-M-18) was grown on barley in Fernbach flasks on a shaker at 30 °C for 10 days.[6] The growth medium was extracted with dichloromethane and filtered, and the filtrate evaporated in vacuo. After the residue was suspended in petroleum ether the pale yellow precipitate (PEI) was collected. The PEI (9.2 g) was chromatographed on a silica gel column (250 g, EM Reagents, 0.063–0.200 mm particle size) with mixtures of hexane–chloroform, chloroform, chloroform–ethyl acetate, and ethyl acetate, to give six crude fractions (fractions I–VI, as determined by TLC, fraction I being eluted first). Fraction III (375 mg) was rechromatographed on silica gel (15 × 1000 mm, 85 g, Woelm, 0.032–0.063 mm particle size) using chloroform–hexane (7:3 v/v) as solvent to give xanthocillin-X dimethyl ether (**9**, 113 mg).

Xanthocillin-X Dimethyl Ether (9). Yellow needles from dichloromethane–hexane: mp 186 °C dec; UV max (95% C_2H_5OH) 238 nm (ϵ 13 600), 297 (12 200), and 364 (46 400); IR ($CHCl_3$) 2122, 1602, 1510, 1260, 1180, and 1031 cm^{-1}; NMR ($CDCl_3$) δ 7.71 (d, 2, J = 9 Hz), 7.01 (s, 1), 6.93 (d, 2, J = 9 Hz), and 3.87 (s, 3); HRMS *m/e* (rel intensity) found 316.11936 (100) (calcd for $C_{20}H_{16}N_2O_2$, 316.12117).

Fraction IV was rechromatographed on silica gel (25 × 1000 mm, 260 g, Woelm, 0.032–0.063 mm particle size) using 10% acetone in benzene as solvent to give five fractions (IVA–IVE). Fraction IVA was rechromatographed by HPLC using 3% 2-propanol in hexane as solvent to give nortryptoquivalone (**7**, faster moving component, 40 mg), and tryptoquivaline (**1**, 200 mg).

Nortryptoquivalone (7). Colorless prisms from dichloromethane–hexane: mp 208–209 °C; $[\alpha]^{25}D$ 255° (c 0.30, $CHCl_3$); UV max (95% C_2H_5OH) 228 nm (sh, ϵ 31 300), 232 (31 800), 253 (sh, 16 900), 278 (7600), 289 (sh, 6500), 304 (5400), and 316 (sh, 4500); IR ($CHCl_3$) 3500, 2960, 2935, 2875, 1783, 1725, 1705, 1672, 1609, 1484, 1469, and 1406 cm^{-1}; NMR ($CDCl_3$) δ 1.27 (d, 3, J = 7 Hz), 1.32 (d, 3, J = 7 Hz), 1.60 (d, 3, J = 7 Hz), 3.10 (d of d, 1, J = 10 and 13 Hz), 3.48 (d of d, 1, J = 10 and 13 Hz), 4.13 (m, 1, J = 7 Hz), 4.36 (q, 1, J = 7 Hz), 5.24 (s, 1), 5.51 (t, 1, J = 10 Hz), 7.12–7.95 (m, 7), and 8.52 (m, 1); HRMS *m/e* (rel intensity) found 488.17037 (100) [calcd for $C_{26}H_{24}N_4O_6$, 488.16958 (M^+)]; CD (95% C_2H_5OH) 256 nm (θ −12 500), 273 (0), 298 (12 000), and 342 (0), estimated absolute error in θ ±2500 deg cm^2/dmol; positive TTC and 2,4-DNP tests.

Tryptoquivaline (1). Colorless prisms from dichloromethane–hexane mixture: mp 155–157 °C; $[\alpha]^{25}D$ 130° (c 0.22, $CHCl_3$); UV max (95% C_2H_5OH) 228 nm (ϵ 41 200), 232 (sh, 40 200), 252 (sh, 19 500), 268 (sh, 10 900), 279 (9500), 307 (3700), and 319 (3000); IR ($CHCl_3$) 3490, 2980, 2940, 2880, 1786, 1728, 1672, 1610, 1485, 1470, and 1410 cm^{-1}; NMR ($CDCl_3$) δ 1.01 (d, 3, J = 7 Hz), 1.13 (d, 3, J = 7 Hz), 1.47 (s, 3), 1.49 (s, 3), 2.14 (s, 3), 2.57 (m, 1), 2.96 (d of d, 1, J = 10 and 13 Hz), 3.16 (d of d, 1, J = 10 and 13 Hz), 4.92 (s, 1), 5.52 (d, 1, J = 9 Hz), 5.63 (t, 1, J = 10 Hz), 7.00–7.78 (m, 7), and 8.67 (m, 1); HRMS *m/e* (rel intensity) found 546.21554 (100) [calcd for $C_{29}H_{30}N_4O_7$, 546.2114 (M^+)]; CD (95% C_2H_5OH) 258 nm (θ −17 500), 282 (0), 305 (7500), and 327 (0), estimated absolute error in θ ±2500 deg cm^2/dmol; positive TTC test.

Fraction IVB was purified by HPLC using 5% 2-propanol in hexane as solvent to give nortryptoquivaline (**2**, 272 mg).

Nortryptoquivaline (2). Colorless prisms from dichloromethane–hexane mixture: mp 256–258 °C; $[\alpha]^{25}D$ 170° (c 0.64, $CHCl_3$); UV max (95% C_2H_5OH) 228 nm (ϵ 43 600), 233 (sh, 42 000), 254 (sh, 18 700), 267 (sh, 11 900), 279 (10 200), 306 (4500), and 319 (3500); IR ($CHCl_3$) 3490, 2980, 2940, 2880, 1790, 1728, 1670, 1610, 1485, 1471, and 1410 cm^{-1}; NMR ($CDCl_3$) δ 1.02 (d, 3, J = 7 Hz), 1.16 (d, 3, J = 7 Hz), 1.58 (d, 3, J = 7 Hz), 2.16 (s, 3), 2.57 (m, 1), 2.94 (d of d, 1, J = 10 and 13 Hz), 3.18 (d of d, 1, J = 10 and 13 Hz), 4.28 (q, 1, J = 7 Hz), 5.10 (s, 1), 5.54 (d, 1, J = 9 Hz), 5.65 (t, 1, J = 10 Hz), 7.01–7.79 (m, 7), and 8.12 (m, 1); HRMS *m/e* (rel intensity) found 532.19525 (100) [calcd for $C_{28}H_{28}N_4O_7$, 532.19580 (M^+)]; CD (95% C_2H_5OH) 254 nm (θ −19 000), 287 (0), 307 (7000), and 325 (0), estimated absolute error in θ ±2500 deg cm^2/dmol; positive TTC test.

Fraction IVC was separated by HPLC using 10% 2-propanol in hexane as solvent to give deoxytryptoquivaline (**3**, faster moving component, 45 mg) and deoxynortryptoquivalone (**8**, 63 mg).

Deoxytryptoquivaline (3). White needles from dichloromethane–hexane mixture: mp 150–152 °C; $[\alpha]^{25}D$ 56.8° (c 0.78, $CHCl_3$); UV max (95% C_2H_5OH) 227 nm (ϵ 44 500), 232 (sh, 41 900), 252 (sh, 18 500), 267 (sh, 12 000), 278 (sh, 10 300), 304 (3300), and 318 (sh, 2700); IR ($CHCl_3$) 3360, 3310, 2980, 2935, 2875, 1790, 1720, 1676, 1604, 1483, and 1469 cm^{-1}; NMR ($CDCl_3$) δ 1.04 (d, 3, J = 7 Hz), 1.20 (d, 3, J = 7 Hz), 1.53 (s, 6), 2.16 (s, 3), 2.54 (m, 1), 3.06 (d, 2, J = 10 Hz), 5.24 (s, 1), 5.52 (d, 1, J = 9 Hz), 5.65 (t, 1, J = 10 Hz), 7.11–7.74 (m, 7), and 8.20 (m, 1); HRMS *m/e* (rel intensity) found 530.21527 (100) [calcd for $C_{29}H_{30}N_4O_6$, 530.21653 (M^+)]; negative TTC test.

Deoxynortryptoquivalone (8). Fine white needles from diethyl ether: mp 192–193 °C; $[\alpha]^{25}D$ 171° (c 0.79, $CHCl_3$); UV max (95% C_2H_5OH) 232 nm (ϵ 32 400), 288 (9250), and 320 (sh, 6250); IR ($CHCl_3$) 3360, 2980, 2935, 2880, 1790, 1705, 1680, 1610, 1588, 1484, and 1469 cm^{-1}; NMR ($CDCl_3$) δ 1.26 (d, 3, J = 7 Hz), 1.30 (d, 3, J = 7 Hz), 1.56 (d, 3, J = 7 Hz), 3.02 (d of d, 1, J = 10 and 13 Hz), 3.32 (d of d, 1, J = 10 and 13 Hz), 4.08 (m, 1, J = 7 Hz), 4.12 (q, 1, J = 7 Hz), 5.36 (s, 1), 5.48 (t, 1, J = 10 Hz), 7.04–7.84 (m, 7), and 8.24 (m, 1); HRMS *m/e* (rel intensity) found 472.17276 (100) [calcd for $C_{26}H_{24}N_4O_5$, 472.17467 (M^+)]; negative TTC test, but positive 2,4-DNP test.

Fraction IVD was purified by chromatography on a silica gel column (85 g, 15 × 1000 mm, Woelm, 0.032–0.063 mm particle size) using 20% ethyl acetate in dichloromethane as solvent to give pure deoxynortryptoquivaline (**4**, 361 mg).

Deoxynortryptoquivaline (4). Colorless prisms from diethyl ether: mp 158–160 °C; $[\alpha]^{25}D$ 69.5° (c 0.82, $CHCl_3$); UV max (95% C_2H_5OH) 228 nm (ϵ 43 900), 233 (sh, 40 100), 254 (sh, 15 600), 268 (11 700), 278 (sh, 10 500), 305 (4100), and 317 (3300); IR ($CHCl_3$) 3360, 2975, 2935, 2880, 1790, 1724, 1676, 1607, and 1483 cm^{-1}; NMR ($CDCl_3$) δ 1.00 (d, 3, J = 7 Hz), 1.15 (d, 3, J = 7 Hz), 1.55 (d, 3, J = 7 Hz), 2.16 (s, 3), 2.58 (m, 1), 2.87 (d of d, 1, J = 10 and 13 Hz), 3.07 (d of d, 1, J = 10 and 13 Hz), 4.12 (q, 1, J = 7 Hz), 5.22 (s, 1), 5.55 (d, 1, J = 9 Hz), 5.65 (t, 1, J = 10 Hz), 7.02–7.74 (m, 7), and 8.14 (m, 1); HRMS *m/e* (rel intensity) found 516.20420 (100) [calcd for $C_{28}H_{28}N_4O_6$, 516.20088 (M^+)]; negative TTC test.

Fraction IVE, which contained several compounds by TLC, was concentrated to about one-third of its original volume. The crystals which appeared were collected and recrystallized from chloroform–hexane to give kotanin (**10**, 900 mg).

Kotanin (10). Cubes: mp >330 °C; $[\alpha]^{23}D$ 40.0 (c 1.65, $CHCl_3$); UV max (95% C_2H_5OH) 236 nm (sh, ϵ 26 900), 253 (sh, 13 600), 296 (sh, 29 000), 308 (33 900), and 318 (sh, 27 600); IR ($CHCl_3$) 3000, 2940, 2850, 1702, 1612, 1590, 1455, and 960 cm^{-1}; NMR ($CDCl_3$) δ 6.73 (s, 1), 5.50 (s, 1), 3.93 (s, 3), 3.80 (s, 3), and 2.73 (s, 3); HRMS *m/e* found 438.13000 (calcd for $C_{24}H_{22}O_8$, 438.13147).

Acetylation of Nortryptoquivaline (2). To a stirred solution of nortryptoquivaline (33 mg, 0.062 mmol) in pyridine (1.5 ml) at 0 °C under an argon atmosphere was added acetic anhydride (1.5 ml). After 5 h at 0 °C, the reaction mixture was poured into cold dilute HCl and extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaCl, dried (anhydrous Na_2SO_4), evaporated in vacuo, and chromatographed (HPCL, 10% ethyl acetate in dichloromethane) to give nortryptoquivaline acetate (**5**, 31.7 mg, 89% yield), colorless prisms from diethyl ether: mp 155–157 °C; $[\alpha]^{25}D$ 159° (c, 0.16, $CHCl_3$); UV max (95% C_2H_5OH) 228 nm (ϵ 41 500), 233 (sh, 39 200), 253 (sh, 19 300), 268 (sh, 12 300), 279 (10 900), 305 (4100), and 319 (3200); IR ($CHCl_3$) 2980, 2940, 2880, 1796, 1730, 1689, 1600, 1485, and 1470 cm^{-1}; NMR ($CDCl_3$) δ 1.04 (d, 3, J = 7 Hz), 1.21 (d, 3, J = 7 Hz), 1.52 (d, 3, J = 7 Hz), 2.16 (s, 3), 2.40 (s, 3), 2.62 (m, 1), 2.98 (d of d, 1, J = 10 and 13 Hz), 3.30 (d of d, 1, J = 10 and 13 Hz), 4.55 (q, 1, J = 7 Hz), 5.39 (s, 1), 5.49 (d, 1, J = 9 Hz), 5.72 (t, 1, J = 10 Hz), 7.20–7.78 (m, 7), and 8.11 (m, 1); HRMS *m/e* no molecular ion, found 514.18434 [calcd for $C_{28}H_{26}N_4O_6$, 514.18523 ($M^+ - CH_3COOH$),[13] 470.19604, calcd for $C_{27}H_{26}N_4O_4$, 470.19540 ($M^+ - CH_3COOH$, $-CO_2$)].

Acetylation of Deoxynortryptoquivaline (4). A stirred solution of deoxynortryptoquivaline (**4**, 35.8 mg, 0.07 mmol) in pyridine (1.5 ml) and acetic anhydride (1.5 ml) under argon was heated to 60–65 °C for 6 h. The reaction mixture was diluted with ethyl acetate and washed with dilute HCl, aqueous $NaHCO_3$, and saturated aqueous NaCl solutions in succession. After drying (anhydrous Na_2SO_4), the organic layer was evaporated in vacuo and chromatographed (HPLC, 15% 2-propanol in hexane) to give crystalline deoxynortryptoquivaline acetamide (**6**, 12.8 mg, 33% yield), recrystallized from dichloromethane–hexane mixture to give colorless prisms: mp 303–304 °C; $[\alpha]^{25}D$ 149° (c 0.27, $CHCl_3$); UV max (CH_3CN) 227 nm (ϵ 36 000), 230 (sh, 28 600), 270 (10 000), 280 (sh, 9400), 305 (3650), and 320 (sh, 2800); IR ($CHCl_3$) 2965, 2930, 2875, 1789, 1735, 1688, 1670, 1600, 1475, and 1468 cm^{-1}; NMR ($CDCl_3$) δ 1.10 (d, 3, J = 7 Hz), 1.22 (d, 3, J = 7 Hz), 1.85 (d, 3, J = 7 Hz), 2.21 (s, 3), 2.46 (s, 3), 3.00 (d of d, 1, J = 10 and 13 Hz), 3.96 (d of d, 1, J = 10 and 13 Hz), 4.78 (q, 1, J = 7 Hz), 5.61 (t,

1, J = 10 Hz), 5.68 (d, 1, J = 9 Hz), 6.41 (s, 1), 7.24–7.84 (m, 7), and 8.24 (m, 1); HRMS m/e (rel intensity) found 558.21118 (100) [calcd for $C_{30}H_{30}N_4O_7$, 558.21145 (M^+)].

Oxidation of Deoxytryptoquivaline (3). To a stirred solution of deoxytryptoquivaline (**3**, 16.7 mg, 0.032 mmol) in dichloromethane (5 ml) at room temperature was added m-chloroperbenzoic acid (m-CPBA, 6.3 mg, 0.037 mmol). The reaction mixture was diluted with dichloromethane (20 ml) after 15 min, and washed with dilute aqueous $NaHCO_3$. The organic layer was dried (anhydrous Na_2SO_4), evaporated in vacuo, and chromatographed (HPLC, 3% 2-propanol in hexane as solvent) to give tryptoquivaline (**1**, 12.6 mg, 73% yield), identical with authentic material.

Oxidation of Deoxytryptoquivalone (8). Deoxynortryptoquivalone (**8**, 19.6 mg, 0.042 mmol) was oxidized with m-CPBA (8.2 mg, 0.048 mmol) to give, after workup and chromatography (HPLC, 3% 2-propanol in hexane), nortryptoquivalone (**7**, 12.6 mg, 62% yield), identical with authentic material.

Oxidation of Deoxynortryptoquivaline (4). Deoxynortryptoquivaline (**4**, 38.4 mg, 0.074 mmol) was oxidized with m-CPBA (14.2 mg, 0.082 mmol) to give, after workup and chromatography (HPLC, 5% 2-propanol in hexane), nortryptoquivaline (**2**, 34.3 mg, 86.6% yield) identical with authentic material.

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Registry No.—**1**, 55387-45-6; **2**, 60676-56-4; **3**, 60676-57-5; **4**, 60676-58-6; **5**, 60676-59-7; **6**, 60676-60-0; **7**, 55387-46-7; **8**, 60676-61-1; **9**, 4464-33-9; **10**, 27909-08-6.

References and Notes

(1) Department of Chemistry.
(2) Department of Nutrition and Food Science.
(3) J. Clardy, J. P. Springer, G. Büchi, K. Matsuo, and R. Wightman, *J. Am. Chem. Soc.*, **97**, 663 (1975).
(4) In ref 3 the second metabolite was called tryptoquivalone. Following a request by Professor Mikio Yamazaki we have changed its name to nortryptoquivalone.
(5) T. Glinsukon, S.-S. Yuan, R. Wightman, Y. Kitaura, G. Büchi, R. C. Shank, G. N. Wogan, and C. M. Christensen, *Plant Foods Man*, **1**, 113 (1974).
(6) A. L. Demain, N. A. Hunt, V. Malik, B. Kobbe, H. Hawkins, K. Matsuo, and G. N. Wogan, *Appl. Environ. Microbiol.*, **31**, 138 (1976).
(7) I. Hagedorn, U. Eholzer, and A. Lüttringhaus, *Chem. Ber.*, **93**, 1584 (1960); A. Takatsuki, S. Suzuki, K. Ando, G. Tamura, and K. Arima, *J. Antibiot.*, **21**, 671 (1968); A. Takatsuki, G. Tamura, and K. Arima, *ibid.*, **21**, 676 (1968); I. Hagedorn and H. Tönjes, *Pharmazie*, **11**, 409 (1956); **12**, 567 (1957).
(8) G. Büchi, D. H. Klaubert, R. C. Shank, S. M. Weinreb, and G. N. Wogan, *J. Org. Chem.*, **36**, 1143 (1971). The fungus was later shown to be *A. clavatus* rather than *A. glaucus* as stated in that paper. We are indebted to Dr. Dorothy Fennell, USDA Fermentation Laboratory, Peoria, Ill., for this identification.
(9) The absolute configuration of the tryptoquivalines could not be determined by the x-ray method and remains unknown.
(10) We wish to thank Professor M. Yamazaki for having compared this metabolite with his norisotryptoquivaline.
(11) M. Yamazaki, H. Fujimoto, T. Kawasaki, E. Okuyama, and T. Kuga, Abstracts, 19th Symposium on the Chemistry of Natural Products, Hiroshima, 1975, p 270; M. Yamazaki, H. Fujimoto, and E. Okuyama, *Tetrahedron Lett.*, 2861 (1976).
(12) G. A. Snow, *J. Chem. Soc.*, 2588 (1954).
(13) None of the hydroxylamine *O*-acetates encountered in this series exhibited a molecular ion peak.

Podophyllotoxin Derivatives. 3.[1] The Remaining Diastereomeric C-4 Alcohols and Ketone of the L Series[2]

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The preparation of L-isopodophyllotoxin (**5**), L-isopicropodophyllin (**7**), and L-isopodophyllotoxone (**12**) is described. With these compounds the eight possible diastereomeric C-4 alcohols (toxins) and four possible C-4 ketones of the L series[2] are all known. Alcohol **5** instead of its epimer **6** was obtained by sodium borohydride reduction of **12** contrary to predictions based on previously reported reduction of DL-**12**.

In the L series, of the eight possible diastereomers of the podophyllotoxin structure (chiral centers at C-2, -3, and -4) and four of the podophyllotoxone structure (chiral centers at C-2 and -3), recent publications[1,3] left two of the alcohols and one ketone undescribed. This is a report of the preparation of these compounds, namely L-isopodophyllotoxin (**5**, 2β,3α,4β), L-isopicropodophyllin (**7**, 2α,3α,4β), and L-isopodophyllotoxone (**12**, 2β,3α) (Chart I).

The Alcohols (Toxins). Compound **5** (2β,3α,4β), previously known only as an unresolved component of a DL mixture,[4] was obtained by inversion at C-4 on treatment of **6** (2β,3α,4α) with dilute acid. Alcohol **6** had been prepared starting with **8** (2α,3α,4α) by an indirect method involving simultaneous epimerization at C-2 and cleavage of the lactone group, and subsequent relactionization of the resulting hydroxy acid (Scheme I).

Additionally **5** was derived from L-isopodophyllotoxone (**12**, see below), by $NaBH_4$ reduction. Finding **5** as the predominant (72%) and sole alcoholic reduction product was unexpected, because it had previously been reported[4] that the DL form of **12** when reduced with $Zn(BH_4)_2$ afforded DL-**6**, the C-4 epimer of DL-**5**.[5] The identity of our reduction product was established by direct spectral (ir, NMR, mass) comparisons of both the alcohol and its acetate with authentic DL samples (prepared by a different route) provided by two laboratories.[6]

Moreover **5** can be reconverted to **6**. The interconversions between **5** and **6** are analogous to the known and fully discussed[7,8] interconversions between alcohols **1** (2α,3β,4α) and **2** (2α,3β,4β). The four compounds constitute the two pairs of half-chair structures made rigid by the 2,3-trans-lactone fusion,[1] with **5** and **6** in the 2β,3α and **1** and **2** in the 2α,3β configurations. Thus the 4-OH groups in both **2** (4β) and **6** (4α), having pseudoaxial conformations, are inverted on treatment with dilute HCl, whereas the corresponding hydroxy groups in **1** (4α) and **5** (4β), being pseudoequatorial, require a two-step process (via the 4-chloro derivative) to complete the inversion.

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