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Structure Determination of Six Fungal Metabolites, Tryptoquivaline E, F, G, H, I and J from *Aspergillus fumigatus*[1]

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The structures of six new tryptoquivaline-related metabolites, tryptoquivaline E—J, isolated from *Aspergillus fumigatus* together with tryptoquivaline C and D were determined.

Keywords——fungal metabolite; tryptoquivaline; structure determination; *Aspergillus fumigatus*; indole compound

The structures of tryptoquivaline C and D(FTC and D) among eight tryptoquivaline-related metabolites isolated together from *Aspergillus fumigatus* have already been determined in our laboratory.[3] The remaining six designated as tryptoquivaline E—J(FTE–J) were considered to have closely related structure to those of FTC and D, since these compounds showed very similar characteristics in the ultraviolet (UV), infrared (IR), mass and nuclear magnetic resonance (NMR) spectra with each other.

Tryptoquivaline E(FTE), mp ~257° (dec.), $[\alpha]_D^{11.5}$ +257°, $C_{22}H_{18}N_4O_5$ (M^+ *m/e* 418) exhibited a positive result in triphenyltetrazolium chloride (TTC) test, suggesting the presence of hydroxylamines in its structure.[4] In the NMR spectrum, a broad singlet which disappeared by treatment with D_2O and by acetylation was actually observed at 10.42 ppm (N–OH). The signals indicating the presence of partial structures as CH_3–CH–, –CH_2–CH–, –CH– and aromatic systems were also observed in the spectrum. On the other hand, signals indicating the presence of a hydroxylated isobutyl group which appeared in the spectra of FTC and D were replaced by a singlet of an isolated aromatic hydrogen. Acetylation of FTE with acetic anhydride in acetic acid afforded an acetate (VII), mp ~200° (dec.), $[\alpha]_D^{17}$ +140°, $C_{24}H_{20}N_4O_6$ (M^+ *m/e* 460). A negative TTC test for hydroxylamines on the acetate indicated that the acetylation occurred possibly on N–OH.

FTE showed a dextro-rotation and positive ORD curve as well as FTC and D. In the NMR spectrum of FTE, the signal pattern of ABX-type hydrogens placed on the five membered spiro-lactone ring closely resembled to those of FTC and D. These facts may strengthen the assumption that the stereochemistry of FTE is possibly similar to that of FTC and D. Thus, the structure of FTE may be proposed as I, including the stereostructure.

Tryptoquivaline F(FTF) was obtained as colorless crystals, mp ~277° (dec.), $[\alpha]_D^{15.5}$—109°, $C_{22}H_{18}N_4O_4$ (M^+ *m/e* 402.1303). Its UV and IR spectra were almost similar to those of FTE. FTF was soluble to a solution of 10% HCl and gave a negative TTC test. The molecular composition of FTF differed from that of FTE by the lack of one oxygen atom. The facts suggested that FTF was a secondary amine but not a hydroxylamine compound. FTF afforded an acetate (VIII), mp 280—283° (dec.), $[\alpha]_D^{12}$ −116°, $C_{24}H_{20}N_4O_5$ (M^+ *m/e* 444.1455) on acetylation in a same manner as in the case of FTE. The acetate showed a methyl signal (3H, singlet) of acetyl group at 2.15 ppm in the NMR and an amide band at 1673 cm^{-1} in the

1) A part of this study was preliminary reported in *Tetrahedron Lett.*, **1976**, 2861.
2) Location: *3-9-1, Izumicho, Narashino, Chiba 275, Japan.*
3) M. Yamazaki, H. Fujimoto, and E. Okuyama, *Chem. Pharm. Bull.* (Tokyo), **25**, 2554 (1977).
4) J. Clardy, J.P. Springer, G. Büchi, K. Matsuo, and R. Wightman, *J. Am. Chem. Soc.*, **97**, 663 (1975); *cf.*, G.A. Snow, *J. Chem. Soc.* **1954**, 2588.

TABLE I. NMR Data of FTE (I), FTF-Ac (VIII), FTG (III), FTH (IV), FTI (V), FTJ (VI), and FTJ–Ac (XI) (δ(ppm) from TMS)

	–CH$_3$				–CH$_2$–		–CH–				Aromatic =CH–			N–OH	N–H
	29 or 30		31 or 32	Ac	13	13	28	15	2	12	5, 6, 7, 8, 21, 22, 23	20	26	16	16
a) In $CDCl_3$															
FTF-Ac			1.68 (d., 7)	2.15 (s.)	3.19 (d.d., 13, 9)	3.33		4.44 (q., 7)	5.65 (s.)	5.40 (t., 9)	7.12—8.07 (7H, m.)	8.21 (1H, d., 7)	8.04 (1H, s.)		
FTI	1.22 (d., 7)	1.28 (d., 7)	1.49 (6H, s.)		3.06 (d.d., 14, 10)	3.39	4.07 (qn., 7)		4.99 (s.)	5.47 (t., 10)	7.00—7.94 (7H, m.)	8.24 (1H, d., 7)		7.01 (s.)	
FTJ-Ac			1.71 (d., 7)	2.22 (s.)	3.28 (d.d., 12, 11)	3.74		4.56 (q., 7)	5.76 (s.)	6.48 (t., 11)	7.30—7.93 (7H, m.)	8.35 (1H, d., 8)	8.75 (1H, s.)		
b) In pyridine-d_5															
FTE			1.63 (d., 7)		3.38 (d.d., 13, 10)	3.56		4.26 (q., 7)	5.42 (s.)	6.48 (t., 10)	6.83—7.80 (7H, m.)	8.17 (1H, d., 8)	8.59 or 8.66[a] (1H, s.)	10.42 (br. s.)	
FTG			1.50 (s.)	1.62 (s.)	3.43 (d.d., 14, 10)	3.64			5.30 (s.)	6.56 (t., 10)	6.96—7.96 (7H, m.)	8.23 (1H, d., 8)	8.66 (1H, s.)	10.64 (br. s.)	
FTH			1.45 (d., 8)		2.96 (d.d., 13, 10)	3.60		3.98 (q., 8)	5.25 (s.)	5.76 (t., 10)	6.68—7.88 (7H, m.)	8.11 (1H, d., 8)	8.38 or 8.41[a] (1H, s.)	10.60 (br. s.)	
c) In dimethylsulfoxide-d_6															
FTE			1.43 (d., 7)		Undefined[b]			4.01 (q., 7)	5.38 (s.)	6.20 (t., 10)	7.17—8.10 (7H, m.)	8.22 (1H, d., 8)	8.57 (1H, s.)	8.52 (br. s.)	
FTG			1.30 (s.)	1.35 (s.)	Undefined[b]				5.16 (s.)	6.16 (t., 10)	7.16—8.04 (7H, m.)	8.20 (1H, d., 7)	8.52 (1H, s.)	8.41 (br. s.)	
FTH			1.48 (d., 6)		Undefined[b]		Undefined[b]		5.46 (s.)	5.62 (t., 10)	7.16—8.08 (7H, m.)	8.22 (1H, d., 6)	8.49 (1H, s.)	8.75 (s.)	
FTJ			1.41 (d., 7)		3.08 (d. -like, 10)	3.10		3.87 (q., 7)	5.41 (br. d., 6)	6.01 (t., 10)	7.20—8.02 (7H, m.)	8.17 (1H, d., 8)	8.49 (1H, s.)		3.76 (br.)

Coupling pattern and/or coupling constant (Hz) of signals are shown in parentheses.
s.: singlet, d.: doublet, t.: triplet, q.: quartet, qn.: quintet, m.: multiplet, br.: broad.
a) Either one of the two is expected of the signal from the solvent.
b) Overlapped with the signal of water in the solvent.

IR spectra. The NMR analysis was performed on the acetate obtained here, since FTF was only slightly soluble in almost all of organic solvents. The signal pattern in the spectrum seemed almost indistinguishable to that of FTE-acetate.

FTF exhibited a levo-rotation contrary to the other tryptoquivalines, *eg.*, FTC, D and E. This compound was supposed therefore to have a different stereostructure from those other tryptoquivalines. FTJ, as will be mentioned later, was found to have the same molecular composition to that of FTF but exhibited a dextro-rotation. Further, FTJ was converted easily into FTF by treatment with alkali. This result indicated that FTF might be a stereoisomer of FTJ. The structure of FTF may be proposed as II.

Tryptoquivaline G (FTG) was crystallized from acetone to give colorless prisms, mp 240—241.5° (dec.), $[\alpha]_D^{11}$ +215°, $C_{23}H_{20}N_4O_5$ (M^+ *m/e* 432.1411). The UV, IR and NMR spectra of FTG also closely resembled those of FTE. From the NMR data, the presence of isobutyl side chain in this compound was excluded. A broad singlet of N–OH which disappeared by treatment with D_2O and by acetylation was observed at 10.64 ppm in the NMR spectrum. Two methyl singlets were newly observed at 1.50 and 1.62 ppm however a doublet of one methyl group and a quartet of a methine hydrogen which appeared in FTE were absent in the spectrum of FTG. Therefore, this compound was supposed to have a geminal dimethyl group at C-15 like FTC. The structure of FTG may be shown as III.

The acetate (IX) of FTG, colorless prisms, mp 231—234°, $[\alpha]_D^{16.5}$ +243°, $C_{25}H_{22}N_4O_6$ (M^+ *m/e* 474) was afforded by acetylation of FTG with acetic anhydride in pyridine.

The stereostructure of FTG was assumed to be closely related to that of FTC since they both exhibited a dextro-rotation and their ORD curves were similar with each other.

Tryptoquivaline H(FTH), mp ~274° (dec.), $[\alpha]_D^{11}$ −155°, $C_{22}H_{18}N_4O_5$ (M^+ *m/e* 418.1272) also exhibited very similar UV and IR spectra comparing with those of FTE. The molecular formula and fragmentation in the mass spectrometry were identical with those of FTE. However, the optical rotation of this compound (−155°) was opposite to that of FTE (+257°). FTH seemed therefore to be a stereoisomer of FTE. Actually, FTH was subsequently obtained by stirring FTE for 10 minutes in a 0.1% KOH methanolic solution at room temperature. It was previously demonstrated that FTC was epimerized in the same condition to give epi-FTC which showed a levo-rotation.[3] Clardy, *et al.* have also reported a similar result on the epimerization of tryptoquivaline with alkali and they have supposed that the epimerization may be due to the change of configuration on the important asymmetric carbon in its molecule.[4] The assumption that FTH is a stereoisomer of FTE may be supported by the facts as above. However, a possibility that FTH would be an artefact derived from FTH during a procedure of isolation is not able to be excluded. By the same reason, FTF is considered to be a possible artefact from FTJ.

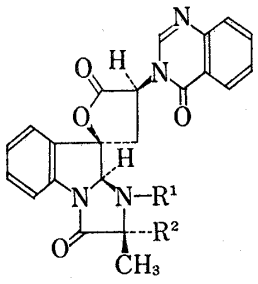
FTH afforded an acetate (X), mp ~223° (dec.), $[\alpha]_D^{15.5}$ −143°, $C_{24}H_{20}N_4O_6$ (M^+ *m/e* 460.1381) on acetylation with acetic anhydride in acetic acid.

Tryptoquivaline I (FTI), colorless leaflets, mp 232—235.5° (dec.), $[\alpha]_D^{14}$ +239°, $C_{27}H_{26}N_4O_6$ (M^+ *m/e* 502), exhibited also very similar IR and NMR spectra to those of the other tryptoquivalines but different in the UV spectrum. In the NMR spectral data of FTI, two doublets of methyl groups at 1.22 and 1.28 ppm and a quintet of methine hydrogen at 4.07 ppm coupled with the methyl groups were observed. Signals of eight aromatic hydrogens were observed in the spectrum but no singlet for an isolated aromatic hydrogen appeared, indicating that this compound contained an isobutyl side chain. However, the chemical shift of the methine quintet was observed abnormally in a lower field (4.07 ppm) comparing with that (2.67 ppm) observed in the spectrum of FTC. Further, no signals for hydrogens attached to the carbon bearing the hydroxyl group were observed. The presence of geminal dimethyl group attached on C-15 was correctly demonstrated as well as in the case of FTC by appearance of a sharp signal at 1.49 ppm (6H, singlet). The UV spectrum of FTI, λ_{max}^{MeOH} nm (ε) 235(31700), 250(21400), 292(9600), 321(6100) characteristically differed from those of general tryptoquiv-

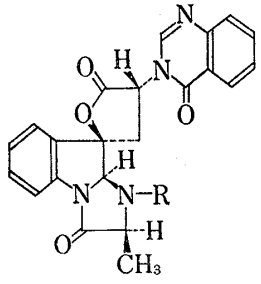
alines but particularly resembled to that of tryptoquivalone which was isolated from *Aspergillus clavatus* by Clardy, *et al.*[4] FTI gave a positive result for TTC test and insoluble to a 10% HCl solution, indicating that this metabolite included hydroxylamine. Thus the structure of FTI may be proposed as V. On acetylation with acetic anhydride in pyridine or acetic acid, FTI has not afforded a single acetate but the formation of three or more products has been demonstrated on the thin layer chromatography. This result may be caused from the unstability of FTI perhaps being due to the presence of a carbonyl group in the isobutyl side chain.

Tryptoquivaline J (FTJ), colorless fine needles, mp 254—258° (dec.), $[\alpha]_D^{14}$ +135°, $C_{22}H_{18}N_4O_4$ (M^+ *m/e* 402), exhibited very similar UV, IR and NMR spectra particularly to those of FTF as already mentioned. FTJ was soluble to a 10% HCl solution as well as FTF. A signal at 3.76 ppm which disappeared by treatment with D_2O and by acetylation was assumed as that of a hydrogen of secondary amine. The acetate of FTJ (XI), mp 254—261° (dec.) obtained by acetylation with acetic anhydride in acetic acid, gave a methyl signal (3H, singlet) at 2.22 ppm of acetyl group in the NMR and an amide band at 1660 cm^{-1} in the IR spectra. The structure of FTJ may therefore be proposed as VI, a stereoisomer of FTF.

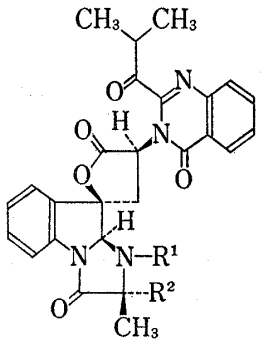
FTJ afforded an epimerization product on stirring for 3 minutes at 55° in a 0.1% KOH methanolic solution and a product was directly identified with FTF. The result indicated



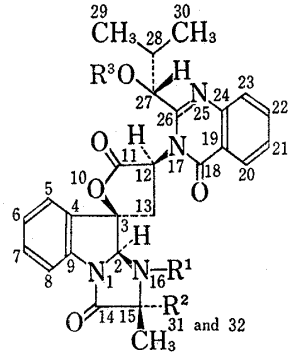
	R^1	R^2
FTE(I)	OH	H
FTE-Ac(VII)	OAc	H
FTG(III)	OH	CH_3
FTG-Ac(IX)	OAc	CH_3
FTJ(VI)	H	H
FTJ-Ac(XI)	Ac	H



	R
FTF(II)	H
FTF-Ac(VIII)	Ac
FTH(IV)	OH
FTH-Ac(X)	OAc



	R^1	R^2
FTI(V)	OH	CH_3
nortryptoquivalone	OH	H
deoxynortryptoquivalone	H	H



	R^1	R^2	R^3
FTC	OAc	CH_3	H
FTD	OAc	H	H
tryptoquivaline	OH	CH_3	Ac
nortryptoquivaline	OH	H	Ac
deoxytryptoquivaline	H	CH_3	Ac
deoxynortryptoquivaline	H	H	Ac

Fig. 1. Structures of Tryptoquivalines

that FTJ and F were isomeric each other as in the case of FTC to epi-FTC, and FTE to FTH. Clardy, *et al.* have demonstrated that dextro-rotatory tryptoquivaline is convertible into epi-tryptoquivaline which shows a levo-rotation by treatment with alkali in D_2O and a hydrogen attached to C-12[5] in the epimerization product has been deuterized.[4] On the other hand, the configuration on C-12 of FTC and D has been determined as S as well as that on C-15 of FTD in our laboratory.[3] It is accordingly assumed that the epimerization of FTC, E and J respectively into epi-FTC, FTH and FTF probably occurred by inversion of the configuration on an important asymmetric carbon, C-12, from S to R.

The novel structure of these fungal metabolites may be biogenetically derived from four amino acids, tryptophan, anthranilic acid, valine and alanine. Deoxynortryptoquivalone, one of four newly isolated tryptoquivaline-related metabolites from *Aspergillus clavatus* by Büchi, *et al.*[6] may be the first compound formed in the tryptoquivaline biosynthesis. Through oxidation of the secondary amine into hydroxylamine, nortryptoquivalone may be formed from the first compound. If the side chain is lost by further oxidation, FTE or FTJ would be formed. On the other hand if reduction of the carbonyl group on the side chain occurs, FTD or nortryptoquivaline would be formed. The geminal dimethyl group at C-15 may be formed by the incorporation of C_1-unit into deoxynortryptoquivalone or the direct participation of methylalanine instead of alanine in the first step of the biosynthesis. Tracer experiment on the biosynthesis of tryptoquivalines is underway in our laboratory at present. The result will be reported elsewhere in near future.

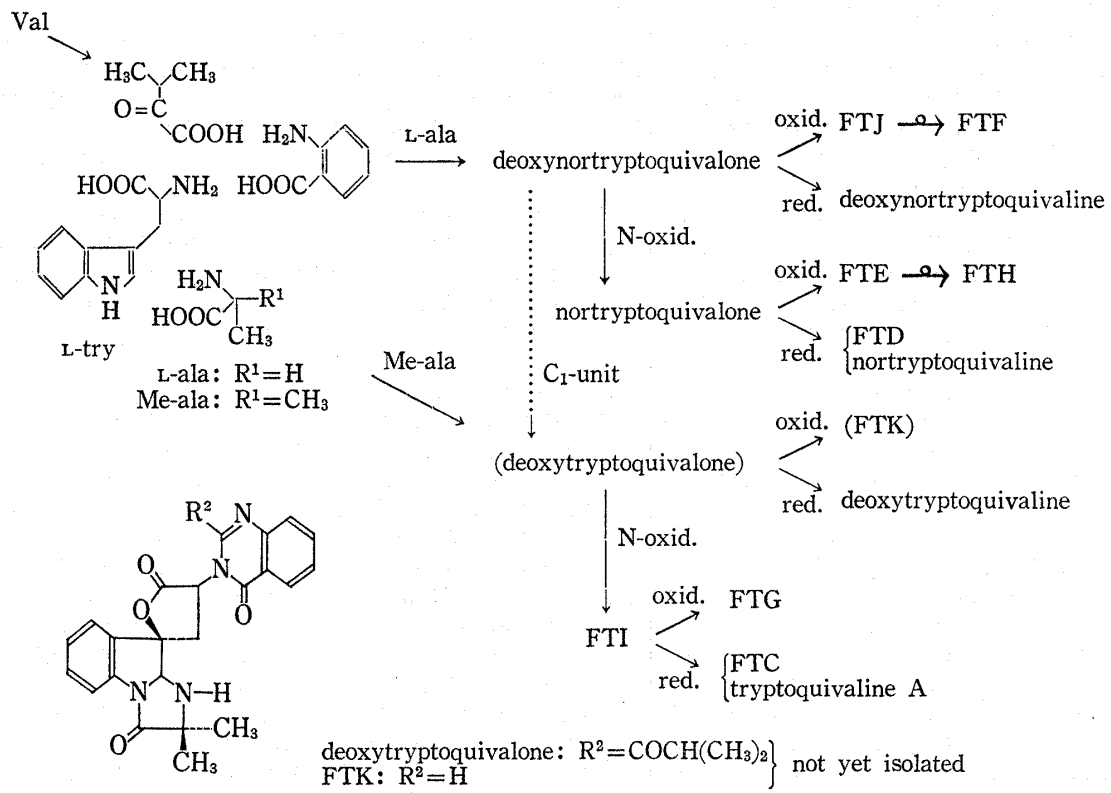


Fig. 2. Hypothetic Scheme for the Tryptoquivaline Biosynthesis

Experimental

All melting points were uncorrected. Optical rotations were measured with Yanagimoto Automatic Polarimeter Model OR-50. UV: Hitachi Recording Spectrophotometer Model 323, IR: Hitachi Grating

5) In the report by Clardy, *et al.* the position of the carbon was indicated as C-19.
6) G. Büchi, K.C. Luk, B. Kobbe, and J.M. Townsend, *J. Org. Chem.*, **42**, 244 (1977).

Infrared Spectrophotometer Model EPI-G3, NMR: Japan Electron Optics Lab. NMR Spectrometer Model JNM-PS-100 (using Me_4Si as internal standard), Mass: Hitachi Double Focus Mass Spectrometer Model RMU-6E and High Resolution Mass Spectrometer Model JMS-01SG-2 of Japan Electron Optics Lab., ORD: Japan Spectroscopic Manufact. Co. ORD/CD Spectropolarimeter Model J-20. Silica gel, E. Merck Kieselgel G nach Stahl was used for thin-layer chromatographic analysis and Kieselgel H for preparative thin-layer chromatography.

Isolation of Metabolites from the Fungus Culture——Culture of *Aspergillus fumigatus* Strain 0011 and isolation of metabolites from the fungus were performed as previously reported.[3]

Tryptoquivaline E: Crystallized from acetone as colorless feather, mp ~257° (dec.), $[\alpha]_D^{11.5}$ +257° (c=0.009, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{18}N_4O_5$ (418.1277), C, 63.15; H, 4.34; N, 13.39. Found: C, 62.88; H, 4.28; N, 13.19. MS: *m/e* (%), 418 (M^+ 100), 402 (94), 356 (22), 254 (37), 215 (86). IR: ν_{max}^{KBr} cm^{-1}, 3430, 1780, 1740, 1732, 1677, 1658, 1614. UV: λ_{max}^{MeOH} nm (ε), 225.5 (32300), 232 (shoulder 29800), 254 (sh. 16000), 265.5 (sh. 11700), 275.5 (sh. 8400), 291 (sh. 3500), 303 (3000), 315 (2600). ORD data (c=0.012, DMSO): $[\alpha]_{589}^{21}$ +242°, $[\alpha]_{550}^{21}$ +242°, $[\alpha]_{500}^{21}$ +258°, $[\alpha]_{450}^{21}$ +355°, $[\alpha]_{400}^{21}$ +548°, $[\alpha]_{350}^{21}$ +855°, $[\alpha]_{320}^{21}$ +1440°.

Tryptoquivaline F: Crystallized from MeOH as colorless fine needles, mp ~277° (dec.), $[\alpha]_D^{15.5}$ −109° (c=0.006, $CHCl_3$). $C_{22}H_{18}N_4O_4$ (402.1328). MS (high resolution): *m/e* (%), 402.1303 (M^+ 50), 374.1350 (22), 215.0799 (47), 146.0605 (100). IR: ν_{max}^{KBr} cm^{-1}, 3365, 1775, 1725, 1664, 1606. UV: λ_{max}^{MeOH} nm (ε), 226 (33400), 232 (sh. 31200), 255 (sh. 12400), 265 (sh. 11100), 276 (sh. 8100), 290 (sh. 3100), 303 (2800), 315 (2200). ORD data (c=0.012, DMSO): $[\alpha]_{589}^{21}$ −119°, $[\alpha]_{550}^{21}$ −119°, $[\alpha]_{500}^{21}$ −203°, $[\alpha]_{450}^{21}$ −305°, $[\alpha]_{400}^{21}$ −559°, $[\alpha]_{350}^{21}$ −1270°, $[\alpha]_{321}^{21}$ −3390°.

Tryptoquivaline G: Crystallized from acetone as colorless prisms, mp 240—241.5° (dec.), $[\alpha]_D^{11}$ +215° (c=0.011, acetone). $C_{23}H_{20}N_4O_5$ (432.1433). MS (high resolution): *m/e* (%), 432.1411 (M^+ 53), 414.1364 (12), 386.1351 (16), 242.0879 (68), 229.0998 (100), 228.1033 (65). IR: ν_{max}^{KBr} cm^{-1}, 3470, 1778, 1738, 1662, 1610. UV: λ_{max}^{MeOH} nm (ε), 226 (34300), 232 (sh. 31700), 253 (sh. 17400), 265 (sh. 11800), 275 (sh. 8200), 291 (sh. 3700), 302 (3000), 315 (2500). ORD data (c=0.015, DMSO): $[\alpha]_{589}^{22}$ +203°, $[\alpha]_{550}^{22}$ +236°, $[\alpha]_{500}^{22}$ +270°, $[\alpha]_{450}^{22}$ +372°, $[\alpha]_{400}^{22}$ +473°, $[\alpha]_{350}^{22}$ +642°, $[\alpha]_{321}^{22}$ +1220°.

Tryptoquivaline H: Crystallized from MeOH as colorless fine needles, mp ~274° (dec.), $[\alpha]_D^{11}$ −155° (c=0.021, acetone). $C_{22}H_{18}N_4O_5$ (418.1277). MS (high resolution): *m/e* (%), 418.1272 (M^+ 20), 400.1166 (7), 215.0812 (47), 146.0498 (100). IR: ν_{max}^{KBr} cm^{-1}, 3430, 1780, 1742, 1734, 1667, 1609. UV: λ_{max}^{MeOH} nm (ε), 226 (33100), 232 (sh. 30900), 255 (sh. 16600), 266 (sh. 11300), 276 (sh. 8500), 291 (sh. 3600), 303 (3100), 315 (2500). ORD data (c=0.018, DMSO): $[\alpha]_{589}^{22}$ −222°, $[\alpha]_{550}^{22}$ −233°, $[\alpha]_{500}^{22}$ −322°, $[\alpha]_{450}^{22}$ −433°, $[\alpha]_{400}^{22}$ −711°, $[\alpha]_{350}^{22}$ −1417°, $[\alpha]_{321}^{22}$ −3944°.

Tryptoquivaline I: Crystallized from CH_2Cl_2–MeOH as colorless leaflets, mp 232—235.5° (dec)., $[\alpha]_D^{14}$ +239° (c=0.16, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{26}N_4O_6 \cdot H_2O$ (502.1852+H_2O), C, 62.30; H, 5.42; N, 10.76. Found: C, 62.22; H, 5.18; N, 10.86. MS: *m/e* (%), 502 (M^+ 28), 486 (19), 252 (20), 242 (32), 229 (100). IR: ν_{max}^{KBr} cm^{-1}, 3480, 1780, 1732, 1710, 1675, 1609. UV: λ_{max}^{MeOH} nm (ε), 235 (31700), 250 (sh. 21400), 292 (9600), 321 (sh. 6100). ORD data (c=0.015, DMSO): $[\alpha]_{589}^{22}$ +236°, $[\alpha]_{550}^{22}$ +270°, $[\alpha]_{500}^{22}$ +338°, $[\alpha]_{450}^{22}$ +507°, $[\alpha]_{400}^{22}$ +743°, $[\alpha]_{350}^{22}$ +2940°, $[\alpha]_{337}^{22}$ +3950°.

Tryptoquivaline J: Crystallized from acetone–MeOH as colorless fine needles, mp 254—258° (dec.), $[\alpha]_D^{14}$ +135° (c=0.024, acetone). *Anal.* Calcd. for $C_{22}H_{18}N_4O_4$ (402.1328), C, 65.66: H, 4.51; N, 13.92 Found: C, 65.54; H, 4.43; N, 13.85. MS: *m/e* (%), 402 (M^+ 59), 215 (24), 202 (23), 174 (71), 44 (100). IR: ν_{max}^{KBr} cm^{-1}, 3375, 1780, 1713, 1670, 1610. UV: λ_{max}^{MeOH} nm (ε), 225.5 (41100), 231 (sh. 38000), 253 (sh. 16200), 264 (sh. 12800), 275 (sh. 9700), 290 (sh. 4200), 302 (3900), 310 (3100). ORD data (c=0.04, DMSO): $[\alpha]_{589}^{21}$ +138°, $[\alpha]_{550}^{21}$ +139°, $[\alpha]_{500}^{21}$ +188°, $[\alpha]_{450}^{21}$ +238°, $[\alpha]_{400}^{21}$ +338°, $[\alpha]_{350}^{21}$ +530°, $[\alpha]_{321}^{21}$ +910°.

Acetylation of Tryptoquivaline E (FTE)——FTE (58 mg, contained a small amount of FTG as a contaminant) was dissolved in a mixture of Ac_2O (0.5 ml) and AcOH (0.5 ml) and poured into water after standing for 15 hr at room temperature. The precipitates formed were collected by filtration, washed with water repeatedly and crystallized from methanol. Recrystallization of crude acetate (15 mg) from acetone afforded colorless leaflets, mp ~200° (dec.), $[\alpha]_D^{17}$ +140° (c=0.01, acetone). *Anal.* Calcd. for $C_{24}H_{20}N_4O_6 \cdot 1/2H_2O$ (460.1383+1/2H_2O), C, 61.40; H, 4.51; N, 11.93. Found: C, 61.10; H, 4.62; N, 11.68. MS: *m/e* (%), 460 (M^+ 2), 418 (7), 400 (14), 254 (100). IR: ν_{max}^{KBr} cm^{-1}, 1778, 1728, 1682, 1610. UV: λ_{max}^{MeOH} nm (ε), 217 (sh. 33300), 225.5 (34100), 232 (sh. 31300), 255 (sh. 15600), 265.5 (sh. 10900), 276 (sh. 8100), 290 (sh. 3600), 302 (2800), 314.5 (2300). NMR: δ (ppm) in *d*-DMSO, 1.42 (3H, doublet, J=7 Hz), 2.30 (3H, singlet), 3.17 (2H, br. doublet J=10), 4.42 (1H, quartet, $J_1=J_2=J_3=7$), 5.80 (1H, singlet), 6.09 (1H, triplet, $J_1=J_2=9$), 7.26—8.01 (7H, multiplet), 8.10 (1H, doublet, J=8), 8.43 (1H, singlet).

Acetylation of Tryptoquivaline F (FTF)——FTF (54 mg, contained a small amount of unknown substance) was dissolved in a mixture of Ac_2O (0.5 ml) and AcOH (0.5 ml) and poured into water after standing for 15 hr at room temperature. White precipitates were collected by filtration, washed with water and crystallized from MeOH. Recrystallization of crude acetate (47 mg) from MeOH afforded colorless needles, mp 280—283° (dec.), $[\alpha]_D^{12}$ −116° (c=0.12, $CHCl_3$), $C_{24}H_{20}N_4O_5$ (444.1432). MS (high resolution): *m/e* (%), 444.1455 (M^+ 100), 402.1323 (12), 373.1266 (9), 257.0943 (42), 228.0886 (23), 215.0817 (23), 202.0739 (35). IR: ν_{max}^{KBr} cm^{-1}, 1787, 1732, 1678 (sh.), 1673, 1608. UV: λ_{max}^{MeOH} nm (ε), 217 (sh. 38900), 226 (37000), 232 (35300), 241 (sh. 25200), 252 (sh. 22500), 265 (21200), 275.5 (sh. 19600), 303 (sh. 5900), 315 (sh. 3100).

Acetylation of Tryptoquivaline G (FTG)——FTG (53 mg) was dissolved in pyridine (0.8 ml). Ac_2O (0.4 ml) was added to the solution. The solution was poured into water under ice cooling after standing for 16 hr at room temperature. The precipitates formed were collected by filtration, washed with water and crystallized from CH_2Cl_2–MeOH. Recrystallization of crude acetate (51 mg) from CH_2Cl_2–MeOH afforded colorless prisms, mp 231—234°, $[\alpha]_D^{16.5}$ +243° (c=0.0095, acetone). *Anal.* Calcd. for $C_{25}H_{22}N_4O_6$ (474.1539) C, 63.28; H, 4.67; N, 11.81. Found: C, 62.98; H, 4.66; N, 11.75. MS: *m/e* (%), 474 (M^+ 27), 432 (100), 414 (75), 386 (43). IR: ν_{max}^{KBr} cm^{-1}, 1786, 1729, 1682, 1616. UV: λ_{max}^{MeOH} nm (ε), 218 (sh. 34400), 226 (35800), 232 (sh. 34100), 255 (sh. 17300), 265 (sh. 12200), 276 (sh. 9200), 290 (sh. 4200), 303 (3300), 315 (2600). NMR: δ (ppm) in *d*-DMSO, 1.37 (3H, singlet), 1.42 (3H, singlet), 2.29 (3H, singlet), 3.13 (2H, br. doublet, J=10), 5.56 (1H, singlet), 6.02 (1H, triplet, $J_1=J_2$=10), 7.12—7.95 (7H, multiplet), 8.05 (1H, doublet, J=8), 8.42 (1H, singlet).

Acetylation of Tryptoquivaline H (FTH)——FTH (49 mg) was dissolved in a mixture of Ac_2O (0.7 ml) and AcOH (0.7 ml) and poured into water under ice cooling after standing for 17.5 hr at room temperature. White precipitates were collected by filtration and washed with water repeatedly. Crude acetate (51 mg) was recrystallized from acetone–MeOH to give colorless needles, mp ~223° (dec.), $[\alpha]_D^{15.5}$ −143° (c=0.0098, acetone), $C_{24}H_{20}N_4O_6$ (460.1381). MS (high resolution): *m/e* (%), 460.1381 (M^+ 15.4), 418.1305 (56.7), 401.1210 (59), 400.1170 (94), 130.0444 (26), 129.0486 (28). IR: ν_{max}^{KBr} cm^{-1}, 1786, 1735, 1666, 1608. UV: λ_{max}^{MeOH} nm (ε), 217 (sh. 30600), 225.5 (32700), 231 (sh. 30900), 254 (sh. 11400), 265 (sh. 9400), 275 (sh. 7700), 290 (sh. 3100), 302 (2600), 315 (2100). NMR: δ (ppm) in *d*-DMSO, 1.44 (3H, doublet, J=8), 2.23 (3H, singlet), 3.06 (1H, d. of d., J_1=14, J_2=10), ~3.50 (1H, undefined), 4.33 (1H, quartet, $J_1=J_2=J_3$=8), 5.56 (1H, triplet, $J_1=J_2$=9), 5.92 (1H, singlet), 7.31—8.07 (7H, multiplet), 8.27 (1H, doublet, J=8), 8.57 (1H, singlet).

Acetylation of Tryptoquivaline J (FTJ)——A solution of FTJ (15 mg) in Ac_2O (0.4 ml) and AcOH (0.4 ml) was allowed to stand for 17.5 hr at room temperature and then poured into water under ice cooling. White precipitates formed were collected by filtration and washed with water repeatedly. Crude acetate (10 mg) was recrystallized from MeOH to give colorless needles, mp 254—261° (dec.), $[\alpha]_D^{10}$ +98° (c=0.123, $CHCl_3$), $C_{24}H_{20}N_4O_5$ (444.1431). MS (high resolution): *m/e* (%), 444.1422 (M^+ 100), 402.1374 (13), 373.1297 (10), 257.0930 (71), 228.0893 (44), 215.0825 (53), 202.0721 (61). IR: ν_{max}^{KBr} cm^{-1}, 1786, 1730, 1681, 1660, 1610. UV: λ_{max}^{MeOH} nm (ε), 225.5 (37200), 231 (sh. 34000), 253 (sh. 13900), 265 (10400), 275.5 (9000), 303.5 (3600), 315 (3000).

Conversion of Tryptoquivaline E into H——FTE (30 mg) was dissolved in a solution of 0.1% methanolic KOH (10 ml) and stirred for 10 min at room temperature. The reaction mixture was poured into water (100 ml) under ice cooling and white precipitates formed were collected by filtration, washed with water repeatedly until washings indicated neutral. The product (17 mg) was crystallized from MeOH and proved to be identical with FTH by comparison of thin-layer chromatograms developed on silica gel plates with $CHCl_3$–acetone (6: 1), $CHCl_3$–MeOH (6: 1) and C_6H_6–acetone (3: 1), IR and NMR spectra. The product showed a levo-rotatory optical activity.

Conversion of Tryptoquivaline J into F——FTJ (10 mg) was dissolved in a solution of 0.1% methanolic KOH (1 ml) and stirred for 3 min at 55°. The reaction mixture was poured into water (5 ml) under ice cooling and white precipitates formed were collected by filtration, washed with water repeatedly. The product (6 mg) was proved to be identical with FTF by comparison of thin-layer chromatograms developed on silica gel plates with $CHCl_3$–MeOH (6: 1), C_6H_6–acetone (5: 2) and AcOEt–EtOH (8: 1), and IR spectrum. The product showed a levo-rotatory optical activity.

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