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Studies on the Constituents of *Picrasma quassioides* BENNET. III.[1] The Alkaloidal Constituents

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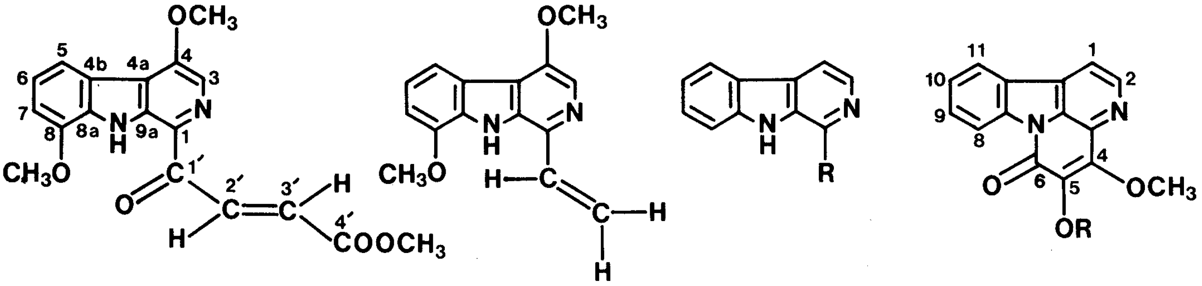
A new β-carboline alkaloid, named picrasidine E (I), was isolated from the wood of *Picrasma quassioides* BENNET (Simaroubaceae) together with seven known alkaloids, 1-methoxycarbonyl-β-carboline (II), 1-ethoxycarbonyl-β-carboline (III), 1-formyl-β-carboline (IV), 1-hydroxymethyl-β-carboline (V), β-carboline-1-propionic acid (VI), 4,5-dimethoxy-canthin-6-one (VII), and 5-hydroxy-4-methoxycanthin-6-one (VIII). These structures were elucidated on the basis of spectroscopic and chemical evidence.

Keywords——*Picrasma quassioides*; Simaroubaceae; wood; picrasidine E; β-carboline type alkaloid; canthin-6-one type alkaloid

In the previous papers,[1,2] we reported the isolation and structural elucidation of five new alkaloids, β-carbolin-1-yl 4,8-dimethoxy-β-carbolin-1-yl-ethyl ketone (named "picrasidine A"), 1-(2-methoxy)ethyl-4,8-dimethoxy-β-carboline (named "picrasidine B"), and 3-methylcanthine-2,6-dione from the root-wood of *Picrasma quassioides* BENNET (Simaroubaceae, Japanese name "Nigaki")[2] and β-carbolin-1-yl 3-(4,8-dimethoxy-β-carbolin-1-yl)-1-methoxypropyl ketone (named "picrasidine C") and 4,9-dimethoxy-1-vinyl-β-carboline (named "picrasidine D") from the wood of this plant.[1] Further chemical examination of the alkaloids of the wood has resulted in the isolation of a new alkaloid (I), named picrasidine E. In addition, we isolated and characterized seven known alkaloids (III—VIII). This paper deals with the isolation and structure elucidation of these compounds.



I IX

II: R = COOCH3
III: R = COOC2H5
IV: R = CHO
V: R = CH2OH
VI: R = CH2CH2COOH

VII: R = CH3
VIII: R = H

Chart 1

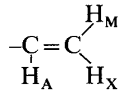
Picrasidine E (I), yellow needles, mp 220 °C, showed a positive Dragendorff test, and its molecular formula was determined as $C_{18}H_{16}N_2O_5$ by high-resolution mass spectrometry (Calcd. *m/z* 340.1059. Found. *m/z* 340.1077). The ultraviolet (UV) spectrum [$\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε):

240 (4.85), 280 (sh, 4.25), 310 (4.35), and 410 (4.35)] was characteristics of a β-carboline type alkaloid,[1,2] and its infrared (IR) spectrum showed absorption bands due to an amino group (3470 cm^{-1}), ester group (1722 and 1290 cm^{-1}), conjugated carbonyl group (1660 cm^{-1}), and ether linkage (1260, 1175, and 1085 cm^{-1}). There were two carbonyl carbon signals at δ 166.36 and 188.81 in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of picrasidine E showed three methoxyl signals at δ 3.88, 4.04, and 4.27, a pair of AX-type proton signals due to *trans* olefin (–CH=CH–) at δ 7.04 and 8.78 (each d, J=16 Hz), and ABX-type proton signals at δ 7.02 (d, J=8 Hz), 7.26 (t, J=8 Hz), and 7.87 (d, J=8 Hz) attributed to 7-H, 6-H, and 5-H, respectively, the last proton being deshielded by the B and C rings. A sharp one-proton singlet at δ 8.22 could be assigned to a lone aromatic proton (3-H), and a lowest field singlet of indolic NH at δ 10.42 which disappeared on treatment with D_2O. From these spectral data, we concluded that picrasidine E has a $-CO-CH=CH-COOCH_3$ grouping. The ^{1}H-NMR and ^{13}C-NMR signals in the aromatic region are very similar to those of 4,8-dimethoxy-1-vinyl-β-carboline (IX),[1] which suggested that picrasidine E has the same disposition as regards the location of two methoxyl groups and the $-CO-CH=CH-COOCH_3$ grouping. The assignment of two aromatic methoxyl signals was confirmed by the ^{1}H–^{1}H two dimensional nuclear Overhauser effect (2D NOE) NMR spectrum of picrasidine E, which showed the presence of NOE between 3-H (δ 8.22) and 4-OCH_3 (δ 4.27) and also 7-H (δ 7.02) and 8-OCH_3 (δ 4.04). On the basis of the above data, picrasidine E should be represented by formula I.

Compounds II—VI all gave a positive Dragendorff test, and their UV spectra exhibited typical absorptions of a β-carboline skeleton.[1,2] Compounds II—VI were identified as 1-methoxycarbonyl-β-carboline,[3] 1-ethoxycarbonyl-β-carboline,[4] 1-formyl-β-carboline,[5] 1-hydroxymethyl-β-carboline,[3] and β-carboline-1-propionic acid,[6] respectively, by direct comparison with synthetic samples [thin-layer chromatography (TLC), IR, spectra, and mixed melting point determination]. This is the first time that compounds V and VI have been isolated from *Picrasma quassioides* BENNET.

Compound VII and VIII gave a positive Dragendorff test, and their UV spectra exhibited typical absorptions of a canthin-6-one skeleton.[7] The ^{1}H-NMR spectrum of VII showed two methoxyl signals at δ 4.09 and 4.49 (each 3H, s), and that of VIII showed a methoxyl signal at δ 4.27 (3H, s). The IR spectrum of VIII showed a band due to a hydroxyl group (3270 cm^{-1}). Compounds VII and VIII were oxidized with $KMnO_4$, affording 1-methoxycarbonyl-β-

TABLE I. ^{1}H-NMR Spectral Data for I and IX

Proton	I	IX	
3H	8.22 (s)		8.03 (s)
5H	7.87 (d, J=8 Hz)		7.86 (d, J=8 Hz)
6H	7.26 (t, J=8 Hz)		7.15 (t, J=8 Hz)
7H	7.02 (d, J=8 Hz)		6.87 (d, J=8 Hz)
NH	10.42 (br s)[a]		9.85 (br s)[a]
2′H	7.04 (d, J=16 Hz)		
3′H	8.78 (d, J=16 Hz)		
		H_A	7.15 J_{AM}=17.5 Hz
		H_M	6.22 J_{AX}=11.0 Hz
		H_X	5.40 J_{MX}=2.0 Hz
4-OCH_3	4.27 (s)		4.08 (s)
8-OCH_3	4.04 (s)		3.92 (s)
4′-OCH_3	3.88 (s)		

I and IX in $CDCl_3$ solution.
a) Disappeared with D_2O.

TABLE II. ^{13}C-NMR Spectral Data for I and IX

Carbon	I	IX
C-1	137.64	134.44
C-3	121.84	120.81
C-4	155.18	151.32
C-4a	119.08	118.96
C-4b	121.19	122.08
C-5	116.12	116.26
C-6	122.18	121.39
C-7	108.15	107.13
C-8	146.19	145.81
C-8a	130.90	134.29
C-9a	130.49	130.02
C-1′	188.81	132.83
C-2′	136.38	116.65
C-3′	130.55	
C-4′	166.36	
4-OCH$_3$	56.61	56.01
8-OCH$_3$	55.66	55.39
4′-OCH$_3$	52.20	

I and IX in CDCl$_3$ solution.

carboline (II). Methylation of VIII with diazomethane gave VII. On the basis of these results, VII and VIII were identified as 4,5-dimethoxycanthin-6-one[8] and 5-hydroxy-4-methoxycanthin-6-one,[9] respectively.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The UV and IR spectra were recorded with Hitachi 340 and Hitachi 260-30 spectrophotometers, respectively. The ^{1}H-NMR spectra were recorded with JEOL JNM-4H-100 and JEOL GX-400 spectrometers, and ^{13}C-NMR spectra were recorded with a JEOL GX-400 spectrometer; chemical shifts are given on the δ scale (ppm) with tetramethylsilane as an internal standard, and coupling constants are given in Hz. The following abbreviations are used: s = singlet, d = doublet, t = triplet, and sh = shoulder. Mass spectra (MS) were measured with a JEOL JMS-01SG-2 mass spectrometer. Column chromatography was carried out on Wako gel C-200 (Wako Pure Chemical Ind., Ltd.). TLC and preparative TLC were performed on precoated Silica gel 60 plates (Merck) and Wako gel B-5 (Wako Pure Chemical Ind., Ltd.), respectively, and the developing solvents were detected with Dragendorff reagent or by UV irradiation.

Extraction——Dried wood (6 kg) of *Picrasma quassioides* collected at the Medicinal Plant Garden, School of Pharmaceutical Sciences, Toho University, in December 1980 was extracted with MeOH (60 l) at 35—40 °C for 48 h. The extract was evaporated to dryness and the residue was partitioned between water and CHCl$_3$. The CHCl$_3$ solution was dried over Na$_2$SO$_4$ and concentrated to give a CHCl$_3$-soluble fraction (90 g) which was passed through a column of silica gel (2 kg). The column was eluted sucessively with CHCl$_3$ and MeOH. 4,5-Dimethoxycanthin-6-one (VII, 3 g) and 5-hydroxy-4-methoxycanthin-6-one (VIII, 25 g) were eluted with CHCl$_3$. Elution with CHCl$_3$–MeOH (19 : 1) afforded 1-methoxycarbonyl-β-carboline (II, 1.0 g) and 1-ethoxycarbonyl-β-carboline (III, 0.5 g), then elution with CHCl$_3$–MeOH (9 : 1) afforded 1-hydroxymethyl-β-carboline (IV, 20 mg), 1-formyl-β-carboline (V, 25 mg), and β-carboline-1-propionic acid (VI, 6 mg).

Picrasidine E (I)——The fraction (690 mg) eluted with CHCl$_3$–MeOH (19 : 1) was repeatedly chromatographed on silica gel and the product was separated by preparative TLC developed with CHCl$_3$, then crystallized from acetone to give picrasidine E (I, 25 mg) as yellow needles, mp 220 °C. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 240 (4.85), 280 (sh, 4.25), 310 (4.35), 410 (4.35). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3465, 2930, 1725, 1660, 1620, 1575, 1565, 1420, 1315, 1290, 1260, 1175, 1085. ^{1}H-NMR and ^{13}C-NMR: Tables I and II, respectively. MS m/z (%): 340 (M$^+$, 32), 282 (23), 281 (100), 266 (20), 251 (7), 228 (5), 223 (5), 212 (3), 197 (2). High-resolution MS, Calcd for $C_{18}H_{16}N_2O_5$, m/z 340.1059. Found, m/z 340.1077.

1-Methoxycarbonyl-β-carboline (II)——Compound II was obtained as colorless needles (acetone), mp 163 °C (lit.,[3] mp 163 °C). MS *m/z*: 226 (M^+). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 240 (4.26), 258 (4.26), 275 (4.31), 301 (4.07), 370 (3.83). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3400, 1685, 1630, 1550, 1469, 1250, 1210, 1075. This compound was identified as 1-methoxycarbonyl-β-carboline by direct comparison with a synthetic sample (TLC, IR spectra, and mixed melting point determination).

Synthesis of II——β-Carboline-1-carboxylic acid (500 mg)[10] was suspended in 5% H_2SO_4 methanol solution, and the mixture was refluxed under moisture-free conditions for 2 h, then cooled in an ice-bath. The reaction mixture was basified with saturated Na_2CO_3 solution. The ester (520 mg) that precipitated was crystallized from acetone, mp 163 °C, MS *m/z*: 226 (M^+). *Anal.* Calcd for $C_{13}H_{10}N_2O_2$: C, 69.02; H, 4.46; N, 12.38. Found: C, 69.35; H, 4.35; N, 12.42.

1-Ethoxycarbonyl-β-carboline (III)——Compound III was obtained as colorless needles (acetone), mp 123 °C.[4] MS *m/z*: 240 (M^+). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 218 (4.90), 247 (4.52), 258 (4.52) 288 (4.56), 302 (4.31), 368 (4.06). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3420, 1682, 1630, 1210, 1180, 1075. This compound was identified as 1-ethoxycarbonyl-β-carboline by direct comparison with a synthetic sample (TLC, IR spectra, and mixed melting point determination).

Synthesis of III——β-Carboline-1-carboxylic acid (500 mg)[10] was suspended in 5% H_2SO_4 ethanol solution, and the mixture was refluxed under moisture-free conditions for 2 h, then cooled in an ice-bath. The reaction mixture was basified with saturated Na_2CO_3 solution. The ester (530 mg) that precipitated was crystallized from acetone, mp 123 °C. MS *m/z*: 240 (M^+). *Anal.* Calcd for $C_{14}H_{12}N_2O_2$: C, 69.70; H, 5.01; N, 11.61. Found: C, 71.05; H, 5.09; N, 11.75.

1-Formyl-β-carboline (IV)——Compound IV was obtained as yellow needles (acetone), mp 202 °C (lit.,[5] mp 198—200 °C). MS *m/z*: 196 (M^+). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 268 (4.93), 304 (4.35), 366 (3.89). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3200, 2880, 1655, 1600, 1335. This compound was identified as 1-formyl-β-carboline by direct comparison with a synthetic sample (TLC, IR spectra, and mixed melting point determination).

Synthesis of IV——Benzylidene harman was prepared from harman[11] and benzaldehyde by the method of Snyder *et al.*[11] Benzylidene harman (200 mg) was dissolved in acetic acid–water (4 : 1) and OsO_4 (25 mg) and *para*-periodate (600 mg) were added. The mixture was stirred for 72 h at room temperature. The solvents were removed under reduced pressure, then the residue was suspended in 10% Na_2CO_3 solution (15 ml) and extracted with $CHCl_3$. The $CHCl_3$ solution was dried over Na_2SO_4, and the solvent was removed under reduced pressure to give a yellow product, which was crystallized from MeOH to give IV (50 mg). MS *m/z*: 196 (M^+). *Anal.* Calcd for $C_{12}H_8N_2O$: C, 73.46; H, 4.11; N, 14.28. Found: C, 73.76; H, 4.18; N, 14.41.

1-Hydroxymethyl-β-carboline (V)——Compound V was obtained as pale yellow needles (acetone), mp 229 °C (lit.,[3] mp 228—230 °C). MS *m/z*: 198 (M^+). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 215 (4.62), 240 (4.70), 285 (4.50), 340 (3.80), 352 (3.80). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3420, 2850, 1630, 1580, 1430. This compound was identified as 1-hydroxymethyl-β-carboline by direct comparison with a synthetic sample (TLC, IR spectra, and mixed melting point determination).

Synthesis of V——A stirred solution of II (500 mg) in THF (20 ml) was treated with $LiAlH_4$. The mixture was stirred for 5 h at room temperature, then excess $LiAlH_4$ was destroyed by addition of ethyl acetate. The resulting solution was extracted with $CHCl_3$. The $CHCl_3$ solution was dried over Na_2SO_4, then concentrated under reduced pressure. The residue was crystallized from MeOH to give IV (280 mg) as pale yellow needles, mp 229 °C. MS *m/z*: 198 (M^+). *Anal.* Calcd for $C_{12}H_{10}N_2O$: C, 74.32; H, 4.23; N, 14.41. Found: C, 74.89; H, 4.34; N, 14.66.

β-Carboline-1-propionic Acid (VI)——Compound VI was obtained as colorless needles ($CHCl_3$–MeOH), mp 215 °C (lit.,[6] mp 216 °C). MS *m/z*: 240 (M^+). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 238 (4.98), 292 (4.51), 336 (4.38), 350 (4.41). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3420, 1680, 1620, 1550, 1390. This compound was identified as β-carboline-1-propionic acid by direct comparison with a synthetic sample (TLC, IR spectra, and mixed melting point determination).

Synthesis of VI——Canthin-6-one[7] (100 mg) in acetic acid (2 ml) was boiled with granulated zinc for 15 min, then the solution was diluted with water, neutralized by addition of 5% Na_2CO_3 solution, and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ and crystallization of the residue from light petroleum ether gave 4,5-dihydrocanthin-6-one (85 mg), which was dissolved in a little MeOH. A mixture of this solution with 5% NaOH solution was warmed at 50 °C, then acidified with acetic acid. The liberated acid (85 mg) was crystallized from water as colorless needles, mp 215—216 °C, (lit.,[6] mp 216 °C). MS *m/z*: 240 (M^+). *Anal.* Calcd for $C_{14}H_{12}N_2O_2$: C, 66.66; H, 4.79; N, 11.10. Found: C, 66.57; H, 4.81; N, 11.19.

4,5-Dimethoxycanthin-6-one (VII)——Compound VII was obtained as pale yellow needles (acetone), mp 146 °C (lit.,[8] mp 145—146 °C). MS *m/z*: 280 (M^+). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 240 (4.54), 248 (4.59), 290 (4.02), 300 (3.99), 349 (3.95), 357 (4.01), 373 (3.92). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1670, 1635, 1270, 1110, 1090. ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.09, 4.49 (each 3H, s, C_4, C_5–OCH_3), 7.50 (1H, t, $J=8$ Hz, 10-H), 7.68 (1H, t, $J=8$ Hz, 9-H), 7.88 (1H, d, $J=5$ Hz, 1-H), 8.06 (1H, d, $J=8$ Hz, 11-H), 8.65 (1H, d, $J=8$ Hz, 7-H), 8.82 (1H, d, $J=5$ Hz, 2-H).

Conversion of 4,5-Dimethoxycanthin-6-one (VII) to 1-Methoxycarbonyl-β-carboline (II)——VII (500 mg) was dissolved in acetone, and oxidized with $KMnO_4$. The resulting product, 1-methoxycarbonyl-β-carboline, was crystallized from acetone to give pale yellow needles (250 mg). The product was identical with the natural product (II) by direct comparison (TLC, IR spectra, and mixed melting point determination).

5-Hydroxy-4-methoxycanthin-6-one (VIII)——Compound VIII was obtained as pale yellow needles (acetone), mp 225 °C (lit.,[9] mp 224—225 °C). MS *m/z*: 266 (M^+). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 246 (4.60), 262 (sh, 4.50), 286 (4.25), 340 (4.01), 356 (4.05), 374 (4.00). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3270, 1670, 1635, 1270, 1100, 1070. ^{1}H-NMR ($CDCl_3$) δ (ppm : 4.27 (3H, s, C_4–OCH_3), 7.48 (1H, t, $J=8$ Hz, 10-H), 7.65 (1H, t, $J=8$ Hz, 9-H), 8.02 (1H, d, $J=5$ Hz, 1-H), 8.10 (1H, d, $J=8$ Hz, 11-H), 8.61 (1H, d, $J=8$ Hz, 7-H), 8.74 (1H, d, $J=5$ Hz, 2-H).

Methylation of 5-Hydroxy-4-methoxycanthin-6-one (VIII)——Methylation of VIII (20 mg) with diazomethane-ether at room temperature for 10 h gave 4,5-dimethoxycanthin-6-one (20 mg), mp 146 °C, MS m/z: 280 (M^+), which was identical with the natural product by direct comparison (TLC, IR spectra, and mixed melting point determination).

Conversion of 5-Hydroxy-4-methoxycanthin-6-one (VIII) to 1-Methoxycarbonyl-β-carboline (II)——VIII (500 mg) was dissolved in acetone, and oxidized with $KMnO_4$. The resulting product, 1-methoxycarbonyl-β-carboline, was crystallized from acetone to give pale yellow needles, mp 168 °C. The product was identical with the natural product by direct comparison (TLC, IR spectra, and mixed melting point determination).

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