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Studies on the Constituents of *Ailanthus altissima* SWINGLE. III.[1] The Alkaloidal Constituents

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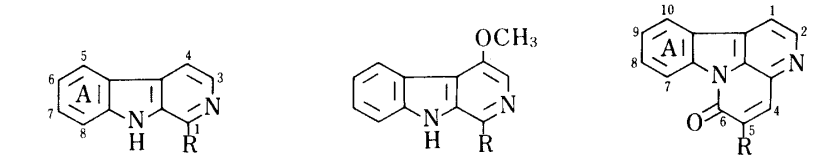
Two New alkaloids, 1-(1-hydroxy-2-methoxy)ethyl-4-methoxy-β-carboline (IV) and 5-hydroxymethylcanthin-6-one (V), have been isolated from the root bark of *Ailanthus altissima* SWINGLE (Simaroubaceae) together with three known alkaloids, β-carboline-1-propionic acid (I), 1-carbamoyl-β-carboline (II), and 1-carbomethoxy-β-carboline (III). The structures were elucidated on the basis of spectral and chemical evidence.

Keywords——*Ailanthus altissima*; Simaroubaceae; β-carboline alkaloid; canthinone alkaloid; 1-(1-hydroxy-2-methoxy)ethyl-4-methoxy-β-carboline; 5-hydroxymethylcanthin-6-one

In the previous paper,[1] the isolation and structural elucidation of three new alkaloids, 1-(2-hydroxy)ethyl-4-methoxy-β-carboline, 1-(1,2-dihydroxy)ethyl-4-methoxy-β-carboline (VI), and 1-methoxycanthin-6-one-3-oxide, and a known alklaoid, 1-acetyl-4-methoxy-β-carboline, from the root-bark of *Ailanthus altissima* SWINGLE (Simaroubaceae, Japanese name "Shinju") were reported. Further chemical examination of the alkaloidal constituents of this plant has resulted in the isolation of two new alkaloids (IV and V). In addition, we have also isolated and characterized three known alkaloids (I—III). This paper deals with the isolation and structural elucidation of these compounds.



I: R=CH_2CH_2COOH
II: R=$CONH_2$
III: R=$COOCH_3$

IV: R=$CH(OCH_3)CH_2OH$
IVa: R=$CH(OCH_3)CH_2OAc$
VI: R=$CH(OH)CH_2OH$
VIa: R=$CH(OAc)CH_2OAc$

V: R=CH_2OH
Va: R=CH_2OAc
VII: R=H

Chart 1

Compounds I—III gave positive Dragendorff tests, and their ultraviolet (UV) spectra exhibited typical absorption of a β-carboline skeleton.[1-3] Compound I was identified as β-carboline-1-propionic acid by direct comparison with a synthetic sample[4] (thin-layer chromatography (TLC), infrared (IR) spectra, and mixed melting point determination). This is the first report of the natural occurrence of I.

Compounds II and III were identified as 1-carbamoyl-β-carboline[5] and 1-carbomethoxy-

TABLE I. ^{1}H-NMR Spectral Data for β-Carboline Alkaloids[a]

Proton	IV	IVa	VI	VIa
3H	8.00 (s)	7.95 (s)	7.89 (s)	8.01 (s)
5H	8.10 (d, J=8.0)	8.08 (d, J=8.0)	8.08 (d, J=8.0)	8.12 (d, J=8.0)
6H	7.28 (t, J=8.0)	7.12 (t, J=8.0)	7.12 (t, J=8.0)	7.17 (t, J=8.0)
7H	7.46 (t, J=8.0)	7.40 (t, J=8.0)	7.39 (t, J=8.0)	7.47 (t, J=8.0)
8H	7.65 (d, J=8.0)	7.56 (d, J=8.0)	7.60 (d, J=8.0)	7.64 (d, J=8.0)
NH	11.17 (br s)	11.30 (br s)	11.35 (br s)	11.75 (br s)
1′H	4.71 (t, J=5.1)	4.39 (dd, J=6.5, 4.5)	4.99 (t, J=5.0)	6.35 (dd, J=8.0, 5.0)
2′H_A	3.75 (d, J=5.1)	4.70 (ABq, J_{AB}=12.0,	3.75 (d, J=5.0)	4.51 (ABq, J_{AB}=12.0,
2′H_B	3.75 (d, J=5.1)	4.98 J_{AX}=4.5, J_{BX}=6.5)	3.75 (d, J=5.0)	4.53 J_{AX}=8.0, J_{BX}=5.0)
C_4–OCH_3	4.10 (s)	4.05 (s)	4.09 (s)	4.10 (s)
$C_{1'}$–OCH_3	3.26 (s)	3.21 (s)		
$C_{1'}$–OAc				1.91 (s)[b]
$C_{2'}$–OAc		2.12 (s)		2.10 (s)[b]

a) Measured in DMSO-d_6 solution; coupling constants in Hz.
b) These assignments may be reversed.

β-carboline,[6] respectively, by direct comparison with synthetic samples (TLC, IR spectra, and mixed melting point determination).

Compound IV, colorless needles, mp 223 °C, showed a positive Dragendorff test. The high-resolution mass spectrum (MS) of IV gave a molecular formula of $C_{15}H_{16}N_2O_3$ (M^+, m/z 272.1161). The UV spectrum of IV exhibited typical absorption of a β-carboline skeleton[1-3] and its IR spectrum suggested the presence of an amino group (3460 cm^{-1}), a hydroxy group (3200 cm^{-1}) and an ether linkage (1260, 1165, and 1072 cm^{-1}). The proton magnetic resonance (^{1}H-NMR) spectrum of IV (Table I) showed signals due to two methoxyl groups (each 3H, s) (an aliphatic methoxyl group at δ 3.26 and an aromatic methoxyl group at δ 4.10), methylene protons at δ 3.75 (2H, d, J=5.1 Hz), and a methine proton at δ 4.71 (1H, t, J=5.1 Hz). The appearance of four aromatic protons at δ 7.28, 7.46, 7.65, and 8.10, and a lowest field broad signal of indolic NH at δ 11.17 which disappeared on exchange with D_2O, in the ^{1}H-NMR spectrum indicated that the A and B rings were unsubstituted.

Acetylation of IV with acetic anhydride–pyridine at room temperature gave the monoacetate (IVa) as colorless needles, mp 125 °C (M^+, m/z 314), and its ^{1}H-NMR spectrum showed the signals of an acetyl group at δ 2.12 (3H, s) and methylene protons at δ 4.70 and 4.98 (AB-type quartets) which showed downfield shifts of *ca.* 1 ppm compared with those of IV. These results suggested that IV has a 1-hydroxy-2-methoxyethyl grouping (–$CH(OCH_3)$–CH_2OH).

The ^{1}H-NMR signals in the aromatic region are very similar to those of VI, which suggested that IV has the same disposition as regards the location of one methoxyl and the 1-hydroxy-2-methoxyethyl grouping. On the basis of these data, compound IV was identified as 1-(1-hydroxy-2-methoxy)ethyl-4-methoxy-β-carboline (the configuration at the C-1 position was not defined).

Compound V, colorless needles, mp 246—247 °C (dec.), showed positive Dragendorff test. The high resolution MS of V gave a molecular formula of $C_{15}H_{10}N_2O_2$ (M^+, m/z 250.0751). The UV spectrum of V exhibited typical absorption of a canthin-6-one,[1,2] and its IR spectrum suggested the presence of a hydroxyl group (3220 cm^{-1}) and a conjugated carbonyl group (1660 cm^{-1}). The ^{1}H-NMR spectrum of V (Table II) showed the signals due to a hydroxymethyl group at δ 4.60 (2H, dd, J=5.7 and 1.2 Hz, –$C\underline{H}_2OH$), which was long-range-coupled with a triplet at δ 8.02 (1H, t, J=1.2 Hz), and δ 5.56 (1H, t, J=5.7 Hz, –$CH_2O\underline{H}$).

TABLE II. ^{1}H-NMR Spectral Data for Canthin-6-one Alkaloids[a)]

Proton	V	Va	VII
1H	8.24 (d, J=5.5 Hz)	7.92 (d, J=5.5 Hz)	8.32 (d, J=5.0 Hz)
2H	8.82 (d, J=5.5 Hz)	8.80 (d, J=5.0 Hz)	8.85 (d, J=5.0 Hz)
4H	8.02 (t, J=1.2 Hz)	8.03 (t, J=1.2 Hz)	8.16 (d, J=10.0 Hz)
5H			7.01 (d, J=10.0 Hz)
7H	8.50 (d, J=8.0 Hz)	8.65 (d, J=8.0 Hz)	8.53 (d, J=8.0 Hz)
8H	7.77 (t, J=8.0 Hz)	7.52 (t, J=8.0 Hz)	7.78 (t, J=8.0 Hz)
9H	7.57 (t, J=8.0 Hz)	7.68 (t, J=8.0 Hz)	7.61 (t, J=8.0 Hz)
10H	8.36 (d, J=8.0 Hz)	8.05 (d, J=8.0 Hz)	8.40 (d, J=8.0 Hz)
C_5–CH_2OH	4.60 (dd, J=5.7, 1.2 Hz)[b)]	5.28 (d, J=1.2 Hz)	
C_5–CH_2O<u>H</u>	5.56 (t, J=5.7 Hz)[b)]		
C_5–CH_2OAc		2.18 (s)	

a) Measured in DMSO-d_6 solution.
b) On addition of D_2O, δ 4.60 (dd) changed to δ 4.60 (d, J=1.2 Hz) and δ 5.56 disappeared.

By replacing the hydrogen of the hydroxyl group at δ 5.56 with D_2O, a doublet of doublets at δ 4.60 was transformed into a doublet (J=1.2 Hz).

Acetylation of V with acetic anhydride–pyridine at room temperature gave the monoacetate (Va) as colorless needles, mp 154 °C (M^+, m/z 292), and its ^{1}H-NMR spectrum showed the presence of an acetyl group at δ 2.18 (3H, s) and methylene protons at δ 5.28 (2H, d, J=1.2 Hz), which showed a downfield shift of 0.68 ppm compared with that of V, the hydroxy group of V at δ 5.56 disappeared on acetylation. The location of the hydroxymethyl group was supported by comparison of the ^{1}H-NMR data (Table II) with those for canthin-6-one (VII).[1)] Chemical shift values of the A and C rings of V were similar to those of VII. These results suggested that the hydroxymethyl group must be attached to the C-5 position. On the basis of these data, compound V was concluded to be 5-hydroxymethylcanthin-6-one.

This is the first time that compounds I—III, and the two new compounds, IV and V, have been isolated from the root-bark of *Ailanthus altissima* SWINGLE.

Experimental

All melting points were determined with a 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 FX-90Q and Hitachi R-900 spectrometers; chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard, and coupling constants (J) in Hz. Abbreviations: s=singlet, d=doublet, dd=doublet of doublets, t=triplet, q=quartet, and br=broad. Mass spectra 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 (prep. TLC) were performed on precoated Silica gel K6 plates (Whatman) and Wako B-5 (Wako Pure Chemical Ind., Ltd.), respectively, and the developing solvents were $CHCl_3$–MeOH (19:1) and $CHCl_3$–MeOH (9:1). The spots were detected with Dragendorff reagent or by UV irradiation.

Extraction——Dried root-bark (10 kg) of *Ailanthus altissima* collected at the Medicinal Plant Garden, School of Pharmaceutical Sciences, Toho University, in December 1981, was extracted with MeOH (60 l) at 40—50 °C for 40 h. The extracts were evaporated to dryness and the residue was partitioned between water and $CHCl_3$. The $CHCl_3$ solution was shaken with 5% H_2SO_4. The aqueous layer was basified with 5% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over Na_2SO_4 and concentrated to give a base fraction (4.0 g), which passed was through a column packed with silica gel. 1-Carbomethoxy-β-carboline (III, 25 mg) was eluted with $CHCl_3$. Elution with $CHCl_3$–MeOH (19:1) afforded 1-carbamoyl-β-carboline (II, 8 mg), and then elution with $CHCl_3$–MeOH (9:1) afforded β-carboline-1-propionic acid (I, 5 mg).

β-Carboline-1-propionic Acid (I)——Compound I was obtained as colorless needles ($CHCl_3$–MeOH), mp 215—216 °C (lit.,[4)] mp 216 °C). MS m/z 240 (M^+). UV λ_{max}^{EtOH} nm (log ε): 238 (4.98), 292 (4.51), 336 (4.38), 350 (4.41). IR ν_{max}^{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 I——Canthin-6-one (VII, 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 sodium bicarbonate, and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ and crystallization of the residue from light petroleum gave 4,5-dihydrocanthin-6-one (88 mg), mp 130 °C (lit.,[4] mp 130 °C). 4,5-Dihydrocanthin-6-one (85 mg) was dissolved in a little MeOH and warmed at 50 °C with 5% sodium hydroxide. The solution was acidified with acetic acid and the liberated acid (85 mg) was crystallized from water as colorless needles, mp 215—216 °C, (lit.,[4] 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.

1-Carbamoyl-β-carboline (II)——Compound II was obtained as colorless needles (MeOH), mp 230 °C (lit.,[5] mp 230 °C). MS *m/z*: 211 (M^+). UV λ_{max}^{EtOH} nm (log ε): 243 (4.31), 253 (4.26), 271 (4.29), 291 (4.04), 361 (3.80). IR ν_{max}^{KBr} cm^{-1}: 3450, 1700, 1620, 1600, 1580, 1490. This compound was identified as 1-carbamoyl-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)[7] was suspended in excess $SOCl_2$, and refluxed at 100 °C under moisture-free conditions for 2 h, followed by removal of the solvent. The reaction mixture was treated with 15% aq. NH_3 (5 ml) at 50 °C for 1 h; on cooling, the amide (356 mg) was precipitated. It was crystallized from MeOH, mp 230 °C (lit.,[5] mp 230 °C). MS *m/z*: 211 (M^+). *Anal.* Calcd for $C_{12}H_9N_3O$: C, 68.24; H, 4.29; N, 19.89. Found: C, 68.97; H, 4.31; N, 19.95.

1-Carbomethoxy-β-carboline (III)——Compound III was obtained as colorless needles (acetone), mp 163 °C (lit.,[6] mp 163 °C). MS *m/z*: 226 (M^+). UV λ_{max}^{EtOH} nm (log ε): 240 (4.26), 258 (4.26), 275 (4.31), 301 (4.07), 370 (3.83). IR ν_{max}^{KBr} cm^{-1}: 3400, 1685, 1630, 1550, 1469, 1250, 1210, 1075. This compound was identified as 1-carbomethoxy-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)[7] was suspended in dry MeOH (30 ml), and the mixture was saturated with dry hydrogen chloride, refluxed under moisture-free conditions for 1 h, and then cooled in an ice-bath. The solid ester hydrochloride precipitate was filtered off, suspended in water, and treated with excess saturated sodium bicarbonate solution. The ester (520 mg) that precipitated was crystallized from acetone, mp 163 °C, (lit.,[6] 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-(1-Hydroxy-2-methoxy)ethyl-4-methoxy-β-carboline (IV)——The fraction (880 mg) eluted with $CHCl_3$–MeOH (9 : 1) was repeatedly chromatographed on silica gel, and the product was separated by prep. TLC with $CHCl_3$–MeOH (9 : 1) and crystallized from acetone to give compound IV (7 mg) as colorless needles, mp 223 °C. UV λ_{max}^{EtOH} nm (log ε): 248 (4.85), 287 (4.45), 336 (3.99), 350 (3.90). IR ν_{max}^{KBr} cm^{-1}: 3460, 3200, 1630, 1600, 1590, 1510, 1455, 1260, 1165, 1072, 750. ^{1}H-NMR: Table I. MS *m/z*: 272 (M^+, 20%), 242 (36), 241 (100), 227 (43), 226 (31), 224 (16), 212 (19), 198 (48), 197 (37), 181 (11), 169 (44), 168 (28). High-resolution MS, Calcd for $C_{15}H_{16}N_2O_3$: *m/z* 272.1161. Found: *m/z* 272.1180.

Acetylation of IV——Compound IV (5 mg) was acetylated with Ac_2O (0.1 ml) in pyridine (0.1 ml). The product (5 mg) was crystallized from acetone to give the monoacetate (IVa) as colorless needles, mp 125 °C. MS *m/z*: 314 (M^+, 21%), 242 (45), 241 (100), 227 (41), 224 (28), 212 (20), 169 (44), 168 (30). IR ν_{max}^{KBr} cm^{-1}: 3460, 1740, 1600, 1500, 1225, 1160, 1070. ^{1}H-NMR: Table I.

5-Hydroxymethylcanthin-6-one (V)——The fraction (300 mg) eluted with $CHCl_3$–MeOH (19 : 1) was repeatedly chromatographed on silica gel, and the product was separated by prep. TLC developing with $CHCl_3$–MeOH (19 : 1), and crystallized from MeOH to give compound V (5 mg) as colorless needles, mp 246—247 °C. UV λ_{max}^{EtOH} nm (log ε): 235 (4.96), 262 (4.36), 270 (4.30), 298 (4.23), 360 (5.51), 376 (4.51). IR ν_{max}^{KBr} cm^{-1}: 3220, 1660, 1630, 1600, 1440, 1390, 1340, 1080, 1040, 740. ^{1}H-NMR: Table II. MS *m/z*: 250 (M^+, 77%), 249 (51), 222 (9), 221 (53), 220 (15), 193 (25), 192 (22), 44 (22), 18 (100). High-resolution MS, Calcd for $C_{15}H_{10}N_2O_2$: *m/z* 250.0751. Found: *m/z* 250.0751.

Acetylation of V——Compound V (2 mg) was acetylated with Ac_2O (0.1 ml) in pyridine (0.1 ml). The product (2 mg) was crystallized from acetone to give the monoacetate (Va) as colorless needles, mp 154 °C. MS *m/z*: 292 (M^+, 25%), 250 (100), 222 (12), 221 (57), 220 (60). IR ν_{max}^{KBr} cm^{-1}: 1740, 1670, 1630, 1570, 1240, 1125, 1040. ^{1}H-NMR: Table II.

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References

1) Part II: T. Ohmoto, K. Koike, and Y. Sakamoto, *Chem. Pharm. Bull.*, **29**, 390 (1981).
2) T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, **30**, 1204 (1982).
3) T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, **31**, 3198 (1983).
4) H. F. Haynes, E. R. Nelson, and J. R. Price, *Austral. J. Sci. Res.*, **A5**, 387 (1952).
5) B. S. Joshi, V. N. Kamat, and D. H. Gawad, *Heterocycles*, **7**, 193 (1977).
6) N. Inamoto, S. Masuda, O. Shimamura, and T. Tsuyuki, *Bull. Chem. Soc. Jpn.*, **34**, 888 (1961).
7) Y. Sung, K. Koike, T. Nikaido, T. Ohmoto, and U. Sankawa, *Chem. Pharm. Bull.*, "accepted."