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Studies on the Constituents of *Ailanthus altissima* Swingle. On the Alkaloidal Constituents

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Canthin-6-one (I), canthin-6-one 3-oxide (III) and new alkaloid, 1-methoxycanthin-6-one (II) were isolated from the wood of *Ailanthus altissima* Swingle (Simaroubaceae). The structure of II was elucidated by spectroscopic studies and chemical evidences.

Ailanthus altissima Swingle is a deciduous tree of Chinese origin belonging to Simaroubaceae which was introduced into Japan in 1887, and is also known as "Tree of Heaven." According to the literature, the bark of this tree has insecticidal property.[2]

The presence of several substances has been reported as follows: quercetin[3] and isoquercetin[4] in the leaves, quassin[5] and ceryl alcohol[6] in the bark, 2,6-dimethoxy-*p*-benzoquinone,[7] ailanthone,[8] chaparrinone,[9] amarolide[10] and acetylamarolide[10] in the wood. The presence of basic substances, however, has not been reported so far.

The authors carried out a chemotaxonomic search for basic components in the wood of *Ailanthus altissima* Swingle and isolated three compounds (Crystal I—III). Crystal I and III are canthin-6-one (I) and its 3-oxide (III), respectively, which are already known compounds, but Crystal III isolated here is the first example of its occurrence in nature. Crystal II is a new compound. The isolation and structure determination of these compounds are reported in the present paper.

Continuous extraction of the dry chips of the wood was carried out with hot ethanol and the basic components were resolved by the method shown in Chart 2.

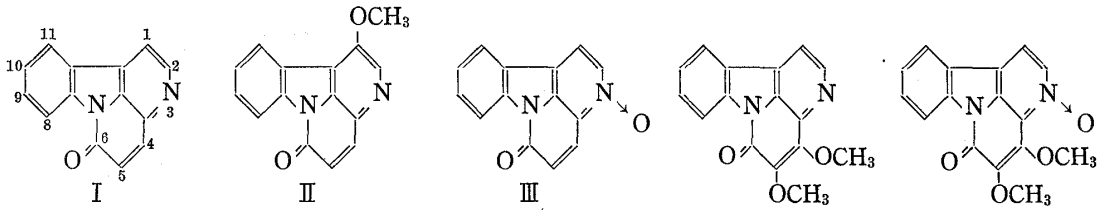

Chart 1

As the basic components showed 5 spots in thin-layer chromatography (referred to as TLC hereinafter), column chromatography with silica gel was used, by which Crystal I and II were isolated. Crystal III was isolated further by preparative TLC.

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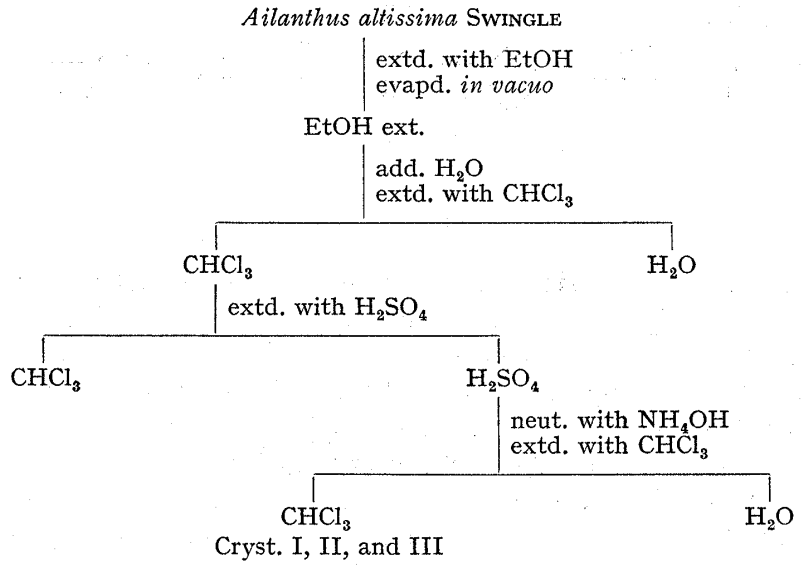

Chart 2

Crystal I was obtained as pale yellow needles with mp 155—156°. The elementary analysis showed the composition of $C_{14}H_8ON_2$ in agreement with M^+ 220 of mass spectrum (MS). Canthin-6-one[11] is assumed to be Crystal I from ultraviolet (UV) and infrared (IR) spectra. This compound is identified by mixed melting point with standard canthin-6-one (I) and by comparison both in IR and TLC.

Crystal II was obtained as pale yellow, fine needles with mp 250—250.5° and was shown to have the molecular formula of $C_{15}H_{10}O_2N_2$, which shows good agreement with the result of M^+ 250 of MS. Maximum absorptions in UV spectrum were found at 279.5, 330, 361.5, 370, and 378.5 nm indicating that this compound is a derivative of I. In IR spectrum, absorptions due to the methoxyl group were observed at 2835, 1119 and 1088 cm^{-1}, and in nuclear magnetic resonance (NMR) spectrum, a 3H singlet was found at 4.25 ppm, and only 7 aromatic and olefinic protons between 6.82 and 8.64 ppm. This compound is, therefore, assumed to have a structure of canthin-6-one with a methoxy substituent.

Although 4-methoxycanthin-6-one[12] from *Charpentiera ovobata* of Amaranthaceae and 5-methoxycanthin-6-one[11,13] from *Zanthoxylum caribaeum* LAM. have been reported, neither of these compounds is identical with Crystal II.

The NMR spectra of Crystal I and II were compared, in which almost no difference was observed in the chemical shift of 6 protons from C_4–H to C_{11}–H. However 1H doublet of 7.82 ppm of I disappeared in Crystal II, and 1H doublet of 8.73 ppm of Crystal I changed to singlet at 8.46 ppm in Crystal II. From these changes in coupling pattern and a shift to a higher magnetic field, it is assumed that the methoxyl group of Crystal II are in the 1- or 2-position.

In order to obtain further proof on the structure of Crystal II, the reactions shown in Chart 3 were carried out according to Nelson's method[14] and a methyl ester (V) with mp 191—192° was obtained as pale yellow columnar crystals. The ester (V) has the molecular formula of $C_{14}H_{12}O_3N_2$ and M^+ in its MS spectrum is found at *m/e* 256. It is assumed that V is methyl β-carboline-1-carboxylate having methoxyl group in the 1- or 2-position, because new absorption of imino group was observed at 3450 cm^{-1} and of ester at 1670, 1275 and 1244 cm^{-1} in IR spectrum, and broad singlet of imino proton at 9.88 ppm, a signal due to ester methyl

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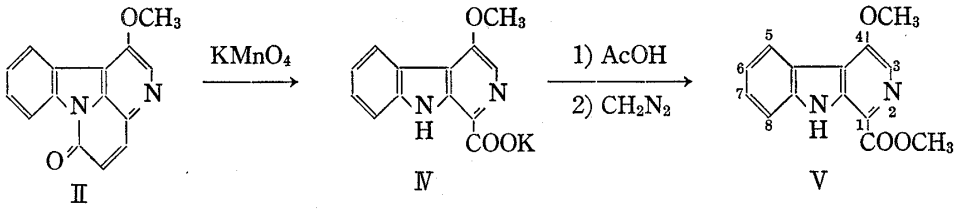

Chart 3

group at 4.11 ppm, and to another signal due to methoxyl group at 4.23 ppm inNMR spectrum.

The NMR spectra of C_3–H of two group of β-carboline type having the same functional group were compared as shown in Table I.

C_3–H of 1-ethyl-4-methoxy-β-carboline (VI)[15]showed a shift to a magnetic field 0.45 ppm higher than that of C_3–H of 1-ethyl-β-calboline (VII),[15] and of V derived from Crystal II showed a shift to a magnetic field 0.34 ppm higher than of C_3–H of the corresponding methyl β-carboline-1-carboxylate (VIII).[16] It is assumed from the above facts that the methoxyl group of compound V is in the 4-position, and consequently, Crystal II is identified with 1-methoxycanthin-6-one.

Table I. NMR Spectral Data[a]

	C_3-H	$\Delta\delta$	C_4-H	C_4-OCH_3
VI	8.00 s		—	4.10 s
		0.45		
VII	8.45 d J=5		7.80 d J=5	—
V	8.21 s		—	4.23 s
		0.34		
VIII	8.55 d J=4.7		8.06 d J=4.7	—

a) chemical shift in (ppm) unit, d: doublet, s: singlet, coupling constant in Hz

Crystal III is a yellow needle crystals with mp 244—245° having the molecular formula of $C_{14}H_8O_2N_2$ showing good agreement with M^+ 236. The UV absorptions were observed at 246.5, 279.5 and 370 nm, and a strong absorption of IR by N-oxide at 1233 cm^{-1}. Canthin-6-one (I) was obtained by catalytic reduction of Crystal III, and canthin-6-one-N-oxide obtained by oxidation of I with *m*-chloroperbenzoic acid was proved to be identical with Crystal III by mixed melting point, IR and TLC.

The melting point of Crystal III was determined to be in good agreement with that of the compound obtained by H.F. Haynes, *et al.*[17] by oxidizing I with hydrogen peroxide. The position of N-oxide in Crystal III, however, remained unknown, and the NMR spectrum of Crystal III was then observed to determine the position of N-oxide.

Table II. NMR Spectral Data[a]

	C_2-H	C_6-H	$\Delta\delta$
Pyridine	8.60 d J=5.0	8.60 d J=5.0	0.50
Pyridine-N-oxide	8.10 d J=5.5	8.10 d J=5.5	
I	8.73 d J=5.5		0.49
III	8.32 d J=6.0		
IX	8.82 d J=5.5		0.49
X	8.33 d J=6.0		

a) chemical shift in δ (ppm) unit, d: doublet, s: singlet, coupling constant in Hz

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16) Y. Kondo and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 837 (1973).
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As shown in Table II, pyridine and 4,5-dimethoxycanthin-6-one were compared with their N-oxides in respect to hydrogen in α-position of nitrogen of these N-oxides. The comparison revealed that a signal of hydrogen in N-oxide shifts to a magnetic field approximately 0.5 ppm higher than its original compound.

On the other hand, C_2–H of Crystal III showed a shift to a magnetic field 0.49 ppm higher than that of C_2–H of I indicating that N-oxide of crystal III is located in 3-position.

Crystal III, therefore, is identified with canthin-6-one-3-oxide.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and uncorrected. UV, IR, NMR, and MS were taken on a Shimazu QV-50, Hitachi EPI-G3, JEOL JNM-4H-100 and JEOL JMS-Ol-SG2 spectrometer, respectively. Gas–liquid chromatography (GLC) was carried out on Hitachi 063 gas–liquid chromatography using a stainless column (3 mm × 1 m) packed with 2%-SE-30 on chromosorb-W (60—80 mesh) with N_2 carrier gas; flow-rate of 30 ml/min. Column chromatography was performed of alumina (Wako, 300 mesh) and silica gel (Wako, C-200). TLC was carried out on silica-B-5, and spots were detected by Dragendorff reagents, $CHCl_3$–AcOEt (4: 1) as a developing solvent.

Extraction and Fractionation of Alkaloids——The dried chipped wood (108 kg) collected in June 1974 at Miyama-cho, Funabashi, Chiba prefecture, were extracted with EtOH at reflux for 24 hr, and the solvent was evaporated under reduced pressure.

The residue (200 g) was added with water and extracted with $CHCl_3$. The $CHCl_3$ solution was shaken with 10% H_2SO_4. The acid layer was made alkaline to about pH 10 with 10% NH_4OH and shaken with $CHCl_3$. The $CHCl_3$ layer, after being washed with water and dried over Na_2SO_4, was evaporated to give a crude alkaloid extracts (8.65 g). The crude alkaloids was chromatographed over alumina and silica gel column.

Canthin-6-one (I)——The fraction (2.65 g) eluted with $CHCl_3$ was crystallized from acetone yielding a crystalline compound (1.05 g) which shows one spot on TLC. The crystals were pale yellow needles, mp 155—156°. UV λ_{max}^{EtOH} nm (log ε) 259 (4.50), 270 (4.38), 300 (4.29), 362 (4.55), 380 (4.52). IR KBr cm^{-1}: 3037, 1677, 1640, 1609, 1449, 1338, 1321, 1311, 1143, 1056. NMR $CDCl_3$ δ: 6.89 (1H, d, J=10 Hz, C_4-H), 7.42 (1H, m, $W_{1/2}$=17 Hz, C_{10}-H), 7.61 (1H, m, $W_{1/2}$=17 Hz, C_9-H), 7.82 (1H, d, J=5.5 Hz, C_1-H), 7.92 (1H, d, J=10 Hz, C_5-H), 7.97 (1H, q, J=8 Hz, C_{11}-H), 8.54 (1H, q, J=8 Hz, C_8-H), 8.73 (1H, d, J=5.5 Hz, C_2-H). Mass Spectrum *m/e* 220 (M^+, base peak), 166, 149, 139, 110, 96, 74, 57. *Anal.* Calcd. for $C_{14}H_8ON_2$: C, 75.67; H, 3.48; N, 12.66. Found: C, 76.36; H, 3.64; N, 12.73.

1-Methoxycanthin-6-one (II)——The fraction (3.85 g) eluted with $CHCl_3$–acetone (8: 1) was crystallized from acetone yielding a crystalline compound (0.62 g) which shows one spot on TLC. The crystals were pale yellow fine needles, mp 250—250.5°. UV λ_{max}^{EtOH} nm (log ε) 279.5 (4.09), 330 (3.87), 361.5 (4.24), 370 (4.01), 378.5 (4.28). IR KBr cm^{-1} 3047, 2937, 2835, 1685, 1635, 1609, 1486, 1449, 1431, 1354, 1331, 1312, 1262, 1226, 1117, 1088, 1003, 832, 793, 747, 607, 465. NMR $CDCl_3$ δ: 4.25 (3H, s, C_1-OCH_3), 6.82 (1H, d, J=10 Hz, C_4-H), 7.42 (1H, m, $W_{1/2}$=14 Hz, C_{10}-H), 7.59 (1H, m, $W_{1/2}$=14 Hz, C_9-H), 7.93 (1H, d, J=10 Hz, C_5-H), 8.15 (1H, d, J=8 Hz, C_{11}-H), 8.46 (1H, s, C_2-H), 8.64 (1H, d, J=8 Hz, C_8-H). Mass Spectrum *m/e* 250 (M^+, base peak), 235, 207, 180, 179, 152, 149, 125, 76, 75, 57. *Anal.* Calcd. for $C_{15}H_{10}O_2N_2$: C, 71.22; H, 3.83; N, 11.30. Found: C, 72.00; H, 4.00; N, 11.27.

Conversion of 1-Methoxycanthin-6-one (II) to Methyl 4-Methoxy-β-calboline-1-carboxylate (V)——A solution of II (100 mg) and $KMnO_4$ (403 mg) in acetone was kept at room temp. for 18 hr. The solvent was evaporated under reduced pressure, and the residue acidified (Congo red) with AcOH and shaken with $CHCl_3$. $CHCl_3$ layer, after being washed with water and dried over Na_2SO_4, was evaporated to give a residue (55 mg). The residue was added with CH_2N_2, and the reaction product was chromatographed over alumina column. The fraction eluted with benzene–$CHCl_3$ (9: 1) was crystallized from benzene yielding (12 mg) of the product which shows one spot on TLC. The crystals were pale yellow needles, mp 191—192°. UV λ_{max}^{EtOH} nm (log ε) 235.5 (4.32), 246.5 (4.29), 266.5 (4.41) 277.5 (4.31), 303 (3.94), 362.5 (3.87). IR KBr cm^{-1} 3349, 3039, 2947, 1608, 1600, 1570, 1490, 1450, 1415, 1350, 1325, 1275, 1245, 1210, 1190, 1138, 1057, 728. NMR $CDCl_3$ δ: 4.10 (3H, s, C_1-$COOCH_3$), 4.23 (3H, s, C_4-OCH_3), 7.21—7.58 (1H × 3, m, C_6, C_7, C_8-H), 8.19 (1H, s, C_3-H), 8.29 (1H, d, J=8 Hz, C_5-H), 9.88 (1H, br. s, NH). Mass Spectrum *m/e* 250 (M^+, base peak), 225, 224, 198, 196, 182, 181, 154, 153, 127, 126, 99. *Anal.* Calcd. for $C_{14}H_{12}O_3N_2$, C, 65.62; H, 4.68; N, 10.93. Found: C, 65.91; H, 4.59; N, 11.02.

Canthin-6-one-3-oxide (III)——The fraction (0.65 g) eluted with $CHCl_3$–acetone (1: 1) was separated on preparative TLC (solvent, $CHCl_3$: AcOEt=5: 1). Crude alkaloids (121 mg) was crystallized from MeOH yielding a crystalline compound which shows one spot on TLC. The crystals was pale yellow needles, mp 244—245° (decomp.). UV λ_{max}^{EtOH} nm (log ε) 246.5 (4.07), 279.5 (4.38), 370 (4.13). IR KBr cm^{-1} 3057, 1677, 1644, 1445, 1428, 1408, 1326, 1315, 1063, 912, 828, 778, 752, 618, 559, 459. NMR $CDCl_3$ δ: 6.92 (1H, d, J=11 Hz, C_4-H), 7.42—7.72 (1H × 2, m, C_9, C_{10}-H), 7.78 (1H, d, J=6 Hz, C_1-H), 7.96 (1H, m, $W_{1/2}$=12 Hz, C_{11}-H),

8.32 (1H, d, $J=6$ Hz, C_2-H), 8.37 (1H, d, $J=11$ Hz, C_5-H), 8.61 (1H, m, $W_{1/2}=12$ Hz, C_8-H). Mass Spectrum *m/e* 236 (M^+, base peak), 220, 208, 192, 191, 181, 179, 166, 165, 164, 153, 149, 140, 138, 114, 76, 75. *Anal.* Calcd. for $C_{14}H_8O_2N_2$: C, 71.18; H, 3.38; N, 11.86. Found: C, 71.29; H, 3.41; N, 12.02.

Conversion of Canthin-6-one 3-Oxide (III) to Canthin-6-one (I)——Solution of III (50 mg) in MeOH (50 ml) was hydrogenated over 10% palladium carbon (10 mg) for 4 hr at room temperature. Removal of the catalyst and solvent left solid which was purified by chromatography to yield a crystalline product. Its crystallization from acetone afforded I, pale yellow needles, mp 154°; mixed mp and all spectral data were identical with these of I.

Conversion of Canthin-6-one (I) to Canthin-6-one 3-oxide (III)——Solution of I (100 mg) in $CHCl_3$ (3 ml) was added to *m*-chloroperbenzoic acid (70 mg) in $CHCl_3$ (2 ml). After 14 hr the reaction mixture was washed with 5% $NaHCO_3$ and H_2O, and $CHCl_3$ layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was crystallized from MeOH to give III (87 mg) as pale yellow needles, mp 244—245°. [literature,[17] mp 237.5—238.5° (decomp.)]. mixed mp and all spectral data were identical with an authentic sample of natural product.

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