

STUDIES ON THE CONSTITUENTS OF THE ROOT-BARK OF *HERNANDIA OVIGERA* L. (III)

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In the course of continuing search for the constituents of the root-bark of *Hernandia ovigera* L., led to the isolation of eight compounds: desoxypodophyllotoxin(I), thalicarpine(III), dehydrothalicarpine(IV), ovigerine(V), hernangerine(VI), hernandone(VIII) and two unknown substances, *mp.* 219–220° and *mp.* 269–270°. Desoxypodophyllotoxin(I) and thalicarpine(III) exhibited a distinctively cytotoxic activity against nasopharynx carcinoma was reported by S.M. Kupchan *et al.*^{8,9}. Dehydrothalicarpine(IV) is merely a dehydro product of thalicarpine(III) at C-6a and C-7 of aporphine moiety. The isolation of dehydrothalicarpine(IV) made the first instance from *Hernandia ovigera* L. Comparison of their components distribution in each part of *Hernandia ovigera* was shown in Table 1.

Previously, we have isolated a tumor-inhibitory active lignan, desoxypodophyllotoxin(I) together with five aporphine alkaloids: hernovine(VII), ovigerine(V), hernangerine(VI), hernandone(VIII) and a new base, 1, 2-methylenedioxy-8, 9-dimethoxy-7H-dibenzo [*de, g*] quinolin-7-one(IX) from the stem-xylem of *Hernandia ovigera*^{1,2}. The present paper is concerned with the isolation of the constituents from the root-bark in order to clarify the occurrence and difference of chemical constituents in each part of the whole plant and aimed to find antitumor components as well. As described in the experimental part, from *n*-hexane and ethanolic extracts, we have isolated eight crystalline substances (Compound A, B, C, D, E, F, G and H).

Compound-(A) was crystallized from methanol as colorless rod-like or feather-like crystals with *mp.* 168–169°, $[\alpha]_D^{25}$ –139.8° (*c*=1.08, CHCl₃). It gave a positive Labat's test. The ultraviolet spectrum with a maximum absorption at 295 nm and its infrared bands at 1770 and 1595 cm⁻¹ indicated the presence of saturated *r*-lactone ring and phenyl group. The *nmr* signal in deuterochloroform appeared three methoxyl protons at 6.18 (6 protons) and 6.25 τ (3 protons), one methylenedioxy protons as singlet at 4.02 τ and four aromatic protons as singlets at 3.65 (2 protons), 3.44 (1 proton) and 3.29 τ (1 proton). From these data, compound-(A) was assumed to be desoxypodophyllotoxin(I) and confirmed by tlc, mixed melting point and ir (nujol) comparison with the authentic sample isolated from the stem-xylem of this plant¹.

Compound-(B) was crystallized from ethylacetate as pale yellow microneedles, *mp.* 150–155°. Thin layer chromatography showed a yellowish brown spot and *R_f* value was 0.5 (SiO₂/CHCl₃-MeOH (8:1)/I₂) resembling that of thalicarpine(III). Its ultraviolet spectrum with the maximum absorptions at 281 and 300 nm and the infrared bands at 2820–2815, 1600, 1500, 1250, 1040, 1020,

1180 and 1120 cm^{-1} indicated the presence of N-methyl, phenyl, ether and methoxyl groups. This base was identified as thalicarpine(III) by tlc, mixed melting point and ir(nujol) comparison with the authentic sample isolated from *Thalictrum dasycarpum*³¹. Thalicarpine(III) has significant inhibitory activity against the walker intramuscular carcinosarcoma 256 in rats^{4a}, and has been selected for clinical evaluation under the auspices of the National Cancer Institute^{4b}.

Compound-(C) was crystallized from methanol as yellow microneedles with mp. 177-179°. Its ultraviolet spectrum with the maximum absorptions at 240, 264 and 334 nm indicated a more highly conjugated chromophore than that of thalicarpine(III)³¹ and resembled closely the spectrum of dehydro-hernandaline⁹. The infrared bands at 1600, 1500, 1245, 1035, 1020, 1170 and 1120 cm^{-1} indicated the presence of phenyl, ether and methoxyl groups. From above data, compound-(C) was assumed to be dehydrothalicarpine(IV) and confirmed by comparison of their tlc, mixed melting point and ir(nujol) with the authentic sample obtained from *Thalictrum dasycarpum*³¹. Dehydrothalicarpine(IV), a new benzyloquinolineaporphine alkaloid, was firstly isolated from *Thalictrum dasycarpum*. It differs from III only in the presence of an additional double bond. The location of the double bond had been elucidated by the cleavage product of the alkaloid with metallic sodium in liquid ammonia by the author⁷.

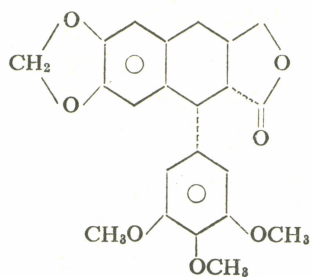
Compound-(D) was a grayish rod-like crystals with mp. 234-236° (MeOH). It gave a positive Labat's test. Its ultraviolet spectrum with maximum absorptions at 234, 269, 277 and 314 nm and the infrared bands at 1595, 1065 and 940 cm^{-1} indicated the presence of phenyl and methylenedioxy groups. These spectral data suggested compound-(D) a 1, 2, 10, 11-tetrasubstituted aporphine base, ovigerine(V)⁸, which was isolated originally from the stem-bark of this plant, and identified by tlc, mixed melting point and ir(KBr) comparison.

Compound-(E) was crystallized as hydrobromide from acetone-methanol with mp. 221-223°(d). It gave a positive Labat's test. The infrared bands at 3480-3460, 1600, 1040 and 940 cm^{-1} indicated the presence of phenolic hydroxyl, phenyl and methylenedioxy groups. Its ultraviolet spectrum with the maximum absorptions at 225, 270 and 310 nm suggested it a 1, 2, 10, 11-tetra-substituted aporphine base⁸. It was found identical with the authentic sample of hernangerine (VI)³² hydrobromide in comparison of their ir(nujol), tlc and mixed melting point.

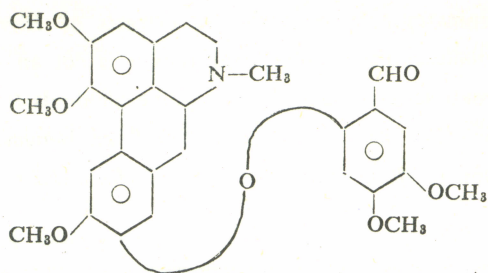
Compound-(F) was a golden needles from chloroform-methanol with mp. 289-291°(d). It formed a red precipitate with mineral acids. The ultraviolet spectrum with the maximum absorptions at 222, 265, 295sh, 314sh, 364 and 423 nm suggested that this base was an oxoaporphine alkaloid¹⁰. Its infrared bands at 1655, 1580, 1070 and 970 cm^{-1} indicated the presence of conjugated carbonyl, phenyl and methylenedioxy groups. It was identified by tlc, mixed melting point and ir(KBr) comparison with the authentic sample of hernandonine(VIII)^{10, 11}.

Compound-(G) was obtained as yellow needles from chloroform-methanol with mp. 219-220° and compound-(H) was crystallized from ethanol as red rod-like crystals, mp. 269-270°(d). The structural elucidation of these two unknown compounds is now in progress.

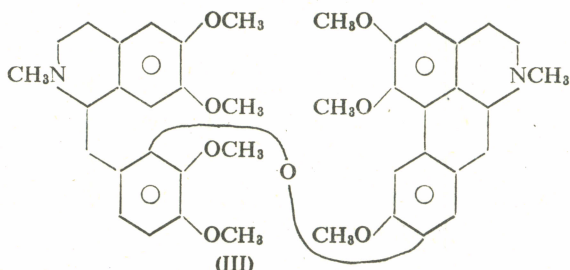
Upon this experiment led to the isolation of two cytotoxic principles, desoxypodophyllotoxin(I) and thalicarpine(III)^{1, 2}. It had been reported that I and III exhibited significant tumor-inhibitory activity against human carcinoma of nasopharynx by S. M. Kupchan *et. al.*^{3, 9}. Thin



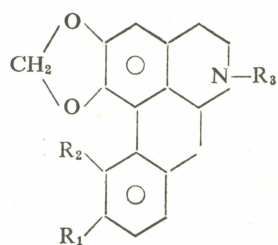
(I)



(II)

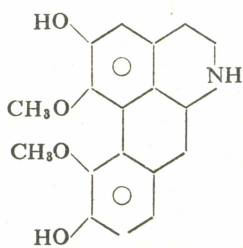


(III)

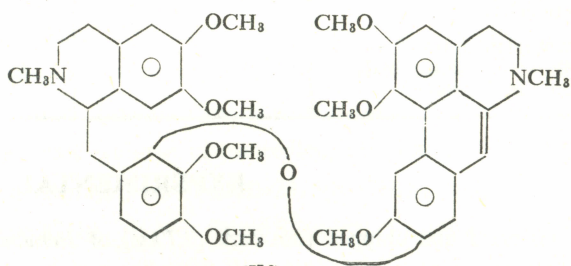


(V) $R_1, R_2 = -O-CH_2-O-$, $R_3 = H$

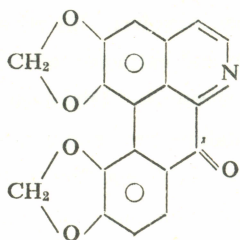
(VI) $R_1 = OH$, $R_2 = OCH_3$, $R_3 = H$



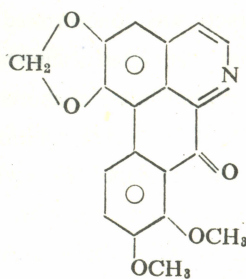
(VII)



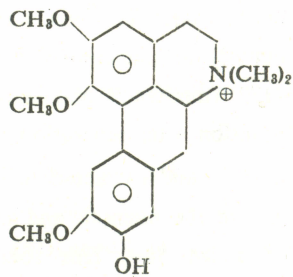
(IV)



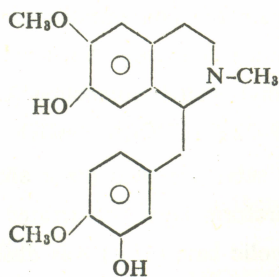
(VIII)



(IX)



(X)



(XI)

layer chromatography of fresh root extracts of *Hernandia ovigera* always show the existence of dehydrothalicarpine(IV) suggesting that it is not an artifact. Evidently base(IV) is a new member of the small series of dehydro-aporphine alkaloids found in nature. The isolation of dehydrothalicarpine(IV) made the first instance from *Hernandia ovigera*. Comparison of the components distribution in stem, xylem and root of *Hernandia ovigera* L. was shown in Table 1.

Table 1.

	stem ⁵⁾	xylem ^{1,2)}	root
desoxypodophyllotoxin(I)		+	+
thalicarpine(III)	+		+
dehydrothalicarpine(IV)			+
ovigerine(V)	+	+	+
hernangerine(VI)	+	+	+
hernovine(VII)		+	
hernandonine(VIII)		+	+
xanthoplanine(X)	+		
reticuline(XI)	+		
1, 2-methylenedioxy-8, 9-dimeihoxy-7H-dibenzo[de, g] qutnolin-7-one(IX)		+	

EXPERIMENTAL

The air-dried and chipped root-bark (2.7 kg.) of *Hernandia ovigera* L. collected in the south of Taiwan in 1968, was refluxed with hot ethanol successively until negative Mayer's test. The total alcohol extracts was concentrated in vacuum to syrupy, then dissolved this residue in 3.5% AcOH, filtered and evaporated somewhat under reduced pressure, and extracted with chloroform. The chloroform solution after evaporation left 40 g. of darkbrown residue. Dissolved this residue in acetone and added 38% HBr solution until acidic, a red crystalline precipitate was obtained. Recrystallization from ethanol to yield compound-(H), 240 mg. with mp. 269-270°(d). After separation of compound-(H), the filtrate made alkaline with conc. ammonia and extracted with chloroform. The organic layer was shaken with 3% NaOH aq. solution to separate the phenolic and nonphenolic bases. The alkaline insoluble layer was washed with water, dried over anhydrous potassium carbonate and concentrated to leave a crude nonphenolic base. This base was subjected to alumina column (5 × 45 cm), eluted with chloroform continuously to give compound-(B) (3 g.), mp. 150-155° (ethylacetate), compound-(C) (50 mg.), mp. 177-179° (methanol) and compound-(G) (77 mg.), mp. 219-220° (CHCl₃ + MeOH). The AcOH acidic solution after separation of chloroform extractable substances was made alkaline with conc. ammonia and extracted with chloroform. The chloroform layer was treated by usual way to separate the phenolic and nonphenolic bases. The phenolic base (37 g.) was dissolved in acetone and added, 38% HBr solution until acidic to yield a crystalline hydrobromide. Recrystallization from actone-methanol gave com-

pound-(E) hydrobromide (2.4 g.), *mp.* 221–223°(d). The nonphenolic base after acid-base treatment was extracted with ether. The ether layer was distilled off the solvent to leave 28 mg. of compound-(D) with *mp.* 234–236° (MeOH).

In order to examine the nonalkaloidal constituents, another batch of 5.5 Kg. root-bark of the same plant, collected in Henchun in 1970, was firstly macerated with *n*-hexane until the organic layer shows nearly colorless. Removal of the solvent left a white crystalline solid. Which was recrystallized from methanol to give compound-(A) (7 g.) with *mp.* 168–169°. The marc of *n*-hexane extract was extracted with hot ethanol exhaustively and the alcohol extract was concentrated under diminished pressure to dryness, then followed the method described above. The acid chloroform extractable nonphenolic portion (11 g.) was chromatographed on silical gel column, eluted with chloroform to yield 47 mg. of compound-(F) with *mp.* 289–291° (CHCl₃ + MeOH). The alkali chloroform extractable phenolic portion (18 g.) was charged with 38% HBr solution until acidic to give a crystalline hydrobromide. Recrystallization from ethanol afforded 1.12 g. of compound-(E) hydrobromide with *mp.* 221–223°(d).

Compound-(A), Desoxypodophyllotoxin(I): as colorless rod-like or feather-like crystals, *mp.* 168–169°(MeOH), $[\alpha]_D^{25}$ –139.8° (*c* = 1.08, CHCl₃), UV $\lambda_{\text{max}}^{\text{EtOH}}$ *nm*(log ϵ): 295(3.71), IR(nujol) *cm*^{–1}: 1770(saturated γ -lactone), 1595(phenyl), 1230, 1125(OCH₃) and 1050, 940(–O–CH₂–O–). NMR (CDCl₃) τ : 6.25, 6.18(9H, 3 $\times$ OCH₃), 4.02(2H, *s*, –O–CH₂–O–) and 3.65, 3.44, 3.29(4H, arom-H). From above data, we assumed compound-(A) to be desoxypodophyllotoxin(I) and showed identical to I by comparison with the authentic sample isolated from the stem-xylem of this plant.

Compound-(B), Thallicarpine(III): as pale yellow microneedles, *mp.* 150–155°(EtOAc), UV $\lambda_{\text{max}}^{\text{EtOH}}$ *nm*(log ϵ): 281(4.30) and 300(4.18), IR(nujol) *cm*^{–1}: 2820–2815(N–CH₃), 1600, 1500(phenyl), 1250, 1040, 1020(=C–O–C), 1125, 1180(OCH₃). It was found identical to thallicarpine(III) by comparison of tlc, mixed melting point and ir(nujol) with the authentic sample isolated from *Thalictrum dasycarpum*.

Compound-(C), Dehydrothallicarpine(IV): as yellow microneedles, *mp.* 177–179°(MeOH), UV $\lambda_{\text{max}}^{\text{EtOH}}$ *nm*(log ϵ): 240sh(4.63), 264(4.72) and 334(4.13), IR(nujol) *cm*^{–1}: 1600, 1500(phenyl), 1245, 1035(=C–O–C), 1170, 1120(OCH₃). This base was identified by comparison of tlc, ir(nujol) and mixed melting point with the authentic sample of dehydrothallicarpine(IV) isolated from *Thalictrum dasycarpum*.

Compound-(D), Ovigerine(V): as grayish rod-like crystals, *mp.* 234–236°(d). It gave a positive Labat's test. UV $\lambda_{\text{max}}^{\text{EtOH}}$ *nm*(log ϵ): 234(4.36), 269(4.15), 272(4.11) and 314(3.82), IR(KBr) *cm*^{–1}: 1595(phenyl) 1065, 940(–O–CH₂–O–). Compound-(D) was identified by ir(KBr), tlc and mixed melting point comparison with ovigerine(V).

Compound-(E), Hernangerine(VI): Its hydrobromide as colorless crystals, *mp.* 221–223°(d) (acetone-methanol). It gave a positive Labat's test. UV $\lambda_{\text{max}}^{\text{EtOH}}$ *nm*(log ϵ): 225(4.54), 270(4.15) and 310(3.81), IR(nujol) *cm*^{–1}: 3480–3460(phenolic-OH), 1600(phenyl), 1040, 940(–O–CH₂–O–). Comparison of their tlc, mixed melting point and ir(nujol) with the authentic sample of hernangerine(VI) hydrobromide confirmed the identification.

Compound-(F), Hernandonine(VIII): as golden needles, *mp.* 289–291°(CHCl₃ + MeOH). It

gave a positive Labat's test and formed a red precipitate in the mineral acid. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm(log ϵ): 222(4.46), 265(4.44), 295sh(3.99), 314sh(3.39), 364(4.22) and 423(4.13), IR(KBr) cm^{-1} : 1655 (conjugated carbonyl), 1580(phenyl), 1070, 970(-O-CH₂-O-). It was identified by tlc, mixed melting point and ir(KBr) comparison with the authentic sample of hernandonine(VIII).

Compound-(G): as yellow needles, mp. 219-220°(CHCl₃+MeOH); Compound-(H): as red rod-like crystals, mp. 269-270°(d)(EtOH). The examination of these two unknown compounds is now in progress.

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