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Studies on the constituents of Hernandia ovigera L.

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A tumor-inhibitor thalicarpine (I), the first dimeric benzylisoquinoline-aporphine alkaloid, had been isolated from the stem-bark of Hernandia ovigera L. (Fam., Hernandiaceae) by M. Tomita et al.[1)-3)]. Further investigation on the constituents of the stem-xylem of this plant, which has not been described, was undertaken for the purpose of examining the antitumor constituents contained.

The extraction and isolation of the constituents from the stem-xylem is described in detail in the experimental part. From the n-hexane and ethanol extracts large amount of a lignan (Compound A) and three alkaloids (Compound B, C and D) were obtained.

Compound A was crystallized from methanol in colorless rod-like crystals, mp 167.5—168.5°, $[\alpha]_D^{29}$ −137° (c=1, $CHCl_3$). It gave positive Labat's test and its uv spectrum in the ethanolic solution showed the absorption maximum at 295 mu (log ε 4.09). The mass spectrum of this compound has a parent peak at m/e 398 corresponding to the molecular formula $C_{22}H_{22}O_7$. The ir bands at 1760 and 1580 cm^{-1} indicated the presence of saturated γ-lactone ring and benzenoid, 1220, 1125, 1050 and 950 cm^{-1} showed the methoxyl and methylenedioxy groups respectively. The nmr spectrum in $CDCl_3$ in which all 22 protons were accounted for, revealed four aromatic protons at 3.39, 3.56 and 3.97τ, two protons of one methylenedioxy moiety as singlet at 4.16τ, nine protons of three methoxyls as singlet at 6.24τ, three methine protons at 6.42—6.78τ and four methylene protons at 7.04—7.48τ. From these data mentioned above, we assumed compound A to be desoxypodophyllotoxin (II). Recently H. Furukawa et al.[4)] reported the isolation of desoxypicropodophyllin (III) from the root-bark of this plant collected in Bonin Island. The relative configurations at C-2, C-3 and C-4 of II is trans-(2:3)-cis-(3:4), whereas that of III is cis-(2:3)-trans-(3:4). Compare key-difference of the physical properties of compound A with desoxypicropodophyllin (III), reported in the literature[5)]., were shown as follow in Table I.

Table 1

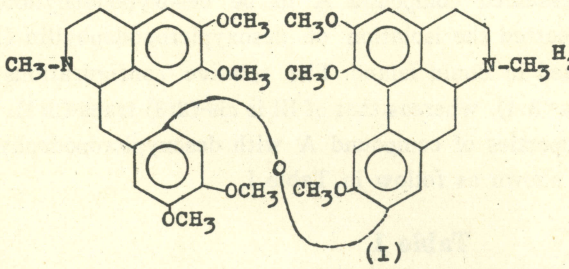
Compound A	Desoxypicropodophyllin (III)
mp 167.5—168.5°(MeOH)	mp 168~170° (EtOH)
$[\alpha]_D^{29}$ −137°($CHCl_3$)	$[\alpha]_D^{30}$ +39° ($CHCl_3$),+42° (pyridine)
ir spectrum in $CHCl_3$ solution: 1). in 1175—1100 cm^{-1} region: two weak and one strong absorption peaks 2). in 1055—1015 cm^{-1} region: two strong absorption peaks and intensities are equal.	only one strong absorption peak in this region. one strong absorption peak at 1055 cm^{-1} and one weak absorption peak at 1015cm^{-1}.

Although we do not have direct comparison of compound A with authentic sample, but the uv, ir and nmr properities of compound A were found identical to desoxypodophyllotoxin (II) in all respects reported in the literature.[5)-10)].

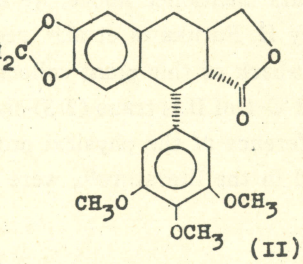
In this experiment we have clarified that desoxypodophyllotoxin (II) is the main component in the stem-xylem of this plant. It was reported by S. M. Kupchan[9)] and Y. Aynehchi[10)] that II, like thalicarpine (I), possessed distinctive cytotoxic activity against cell derived from human carinoma of the Nasopharynx in the tissue culture (KB). The isolation of desoxypodophyllotoxin (II) made the first instance from the stem-xylem of Hernandia ovigera L.

Compound B was crystallized from methanol in plates from the alkaline phenolic fraction, mp. 241—243°, $[\alpha]_D^{28}$ +226° (c=0.8, methanol), it gave positive ferric chloride and negative Labat's and Gibb's tests. Its uv spectrum in the ethanolic solution showed the following absorption maxima at 222 (4.46), 272 (4.33) and 306 mu (log ε 3.98). The ir bands at 1600, 1120 and 1030 cm^{-1} indicated the presence of benzenoid and methoxyl groups. The nmr in trifluoroacetic acid solution exhibited a singlet at 6.85τ representing six protons of two methoxyl groups, multiplets at 3.29 and 3.38τ representing three aromatic protons. No signal related to C—11 and N—methyl protons was detected. From the data described above, compound B was suspected to be a 1, 2, 10, 11-tetrasubstituted secondary aporphine base and was found identical to authentic hernovine(IV)[11)12)] by direct comparisons of their ir, tlc and mixed melting point.

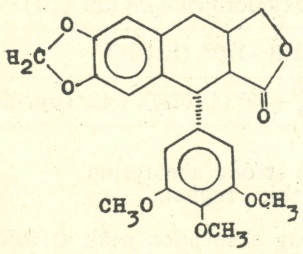
The acetic acid chloroform soluble nonphenolic portion was chromatographed through a alumina column and compound C, a yellow needle crystal, mp. 213—215°, and compound D, mp. 287—290°, were obtained. The structural elucidation of these two compounds are now in progress.



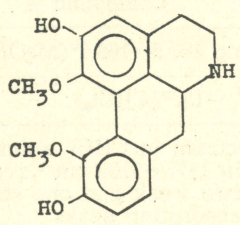
(I)



(II)



(III)



(IV)

Experimental

All melting points were measured by use of the Yanagimoto Micromelting Point Apparatus and incorrected. Optical rotation was determined on a Rex Photoelecric Polarimeter, model NEP—2. IR spectra were recorded on a Hitachi Grating Infrared Spectrophotometer, model EPI—G2. UV absorption spectra were taken on a Hitachi Double Beam 124 Spectrophotometer. The nmr spectra were measured in $CDCl_3$ using $(CH_3)_4$ Si as the internal standard and in CF_3COOH, $(CH_3)_4$ Si as the external reference.

Extraction and Isolation:

The air-dried and crushed stem-xylem (13 Kg.) of Hernandia ovigera L. collected in Hengchun Peninsula, was macerated with portions of n-hexane until the organic layer was almost colorless. The n-hexane extracts were combined and concentrated. A crystalline substance, compound A was obtained. It was crystallized from methanol to give colorless rod-like crystals (20 g., yield 0.15%).

Upon the completion of the n-hexane extraction, the marc was extracted with portions of hot ethanol until the extract was negative to Mayer's test. The total ethanolic extract was concentrated under reduced pressure to give a dark brown syrupy residue (800 g.). This residue was dissolved in 5% AcOH solution and filtered. The insoluble substance was discarded. The acidic solution was concentrated its volume to ten liters and extracted with chloroform. The chloroform extract after usual acid-base treatment was shaken with 3% NaOH solution to separate the phenolic and nonphenolic bases. The Chloroform layer was washed with water, dried over anhydrous potassium carbonate and evaporated to afford a mixture of nonphenolic bases (15 g.). This crude alkaloids was chromatographed on alumina column (4×28 cm) (Wako active alumina, 300 mesh), eluted with n-hexane, n-hexane-$CHCl_3$, $CHCl_3$, $CHCl_3$-MeOH (20:1), $CHCl_3$-MeOH (1:1) and MeOH successively. The n-hexane-$CHCl_3$ eluate was evaporated to give a yellow residue, which was crystallized from ethanol to yield a yellowish needle crystals (30 mg.), mp. 213—215° (compound C). The $CHCl_3$ and $CHCl_3$-MeOH (20:1) eluates were combined and evaporated to leave a yellow residue, which was crystallized from $CHCl_3$ to yield a golden needle crystals (104 mg.), mp. 287—290° (compound D). The NaOH layer was made ammonia alkaline with ammonium chloride and extracted with $CHCl_3$. After washing with water and drying over anhydrous magnesium sulfate, the chloroform extract was evaporated to give a crude phenolic base (13.3 g.). The remained acetic acid solution after $CHCl_3$ extraction was made alkaline with ammonia and extracted with $CHCl_3$. The $CHCl_3$ extract was treated as described above to separate the nonphenolic base (2.2 g.) and phenolic base. The phenolic solution after distilling off the solvent yielded 600 mg. of crude crystals, which was recrystallized from methanol to give white plates (410 mg.), mp. 241—243° (compound B). The mother liquid, evaporated to leave a dark residue (5.5 g.) is still under investigation.

Compound A: mp. 167.5—168.5° (MeOH), as colorless rod-like crystals, gave a positive Labat's test, $[\alpha]_D^{29}$ −137° (c=1, $CHCl_3$); mass spectrum: M^+m/e 398 (corresponding to $C_{22}H_{22}O_7$); ir (KBr) cm^{-1}: 1760 (saturated γ-lactone), 1580 (benzenoid), 1220, 1125 (—OCH_3) and 1050, 925 (methylenedioxy); uv λ_{max}^{EtOH} mu (log ε): 295 (4.09); nmr ($CDCl_3$) τ: 3.39, 3.56 and 3.79 (4H, aromatic protons), 4.16 (2H, s, methylenedioxy protons), 6.24 (9H, s. three methoxyl protons), 6.72—6.78 (3H, methine protons) and 7.04—7.48 (4H, methylene protons). Its properties with desoxypodophyllotoxin (II) reported in the litearture[5] were found identical.

Compound B: mp. 241—243° (MeOH), $[\alpha]_D^{28}$ +226° (c=0.8, MeOH), as white plates, sparingly soluble in MeOH, EtOH, $(CH_3)_2CO$ and $CHCl_3$, but soluble in warm MeOH and EtOH. It gave a positive ferric chloride test and negative Gibbs' and Labat's tests. UV λ_{max}^{EtOH} mμ (log ε): 222

(4.46), 272 (4.33) and 306 (3.98); ir (KBr) cm^{-1}: 1600 (benzenoid), 1120 and 1030 (—OCH_3); nmr (CF_3COOH)τ: 3.29, 3.38 (3H, aromatic protons) and 6.85 (6H, s. two methoxyl protons). Its ir (nujol), tlc and mixed melting point were identical with those of hernovine (IV).

Compound C and Compound D: mp. 213—215° and 287—290° were unknown bases. Their physical and chemical properties and structural elucidation are now in progress.

Acknowledgement

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References

1). M. Tomita, H. Furukawa, S-T Lu, S.M. Kupchan: Tetrahedron Letters, **48** 4039 (1965)
2). M. Tomita, H. Furukawa, S-T Lu, S.M. Kupchan: J. Chem. Pharm. Bull. (Tokyo), **15** 959 (1967)
3). M. Tomita, S-T Lu, Y-Y Chen: Yakugaku Zasshi, **86** (9) 764—765 (1966)
4). H. Furukawa, F. Ueda, M. Ito, K. Ito, H. Ishi, J. Haginiwa: Yakugaku Zasshi, **92** (2) 150—154 (1972)
5). A.W. Schrecker, J. L. Hartwell: J. Am. Chem. Soc., **75** 5916 (1953)
6). K. Noguchi: Yakugaku Zasshi, **60** 629 (1940)
7). C. Hata: J. Chem. Soc. (Japan), **63** 1540 (1942)
8). J.L. Hartwell et al.: J. Am. Chem. Soc., **74** 4470 (1952)
9). S. M. Kupchan, R. H. Hemingway, J. C. Hemingway: J. Pharm. Sci., **36** (3) 408—409 (1967)
10). Y. Aynehchi: J. Pharm. Sci., **60** (1) 121 (1971)
11). M. P. Cava, K. Bessho, B. Douglas, S. Markey, R. F. Raffauf and J. A. Weibach: Tetrahedron Letters, **15** 1577—1581 (1966)
12). S-T Lu and T-L Su: J. Chinese Chem. Soc., **20** 75 (1973)

Summary

A tumor-inhibitor thalicarpine (I), the first dimeric benzylisoquinoline-aporphine-alkaloid, had been isolated from the stem-bark of Hernandia ovigera L. (Family, Hernandiaceae) by M. Tomita et al.[1-3]. Further investigation on the constituents of the stem-xylem of this plant, which had not been reported, was undertaken, aimed at the isolation of other antitumor constituents.

Desoxypodophyllotoxin (II) and hernovine (IV) were isolated and identified by comparison of their physical and chemical properties with those of II described in the literature[5]

and authentic sample of hernovine (IV). Two other unknown bases, mp. 213—215° and 287—290° respectively were also isolated. The structural elucidations of these two bases are now in progress.

Desoxypodophyllotoxin (II) was found to be the main component in the stem-xylem of this plant and like thalicarpine (I) possessing distinctive cytotoxic activity reported by S. M. Kupchan et al.[9]

中 文 摘 要

蓮葉桐 (Hernandia ovigera L.) 之成分研究

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抗癌性植物蓮葉桐 (Hernandia ovigera L.) 之心材部經抽取，分離結果得四種結晶性物質，II爲 mp. 167.5～168.5°C (MeOH), $[\alpha]_D^{29}$ −137° ($CHCl_3$)，其物理化學諸恆數與文獻上所載的 desoxypodophyllotoxin 相符而認爲 desoxypodophyllotoxin (II). IV 爲 mp. 241～243° (MeOH), 經與標品直接比較而證明爲 hernovine (IV) 其他二種爲未知鹽基， mp. 213～215° 及 287～290° ，其構造之決定今仍在進行中。

從本研究得知 desoxypodophyllotoxin (II) 爲本植物心材部之主成分，且近年 S.M. Kupchan等報告具顯著之細胞毒作用。