

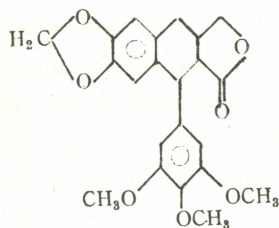
STUDIES ON THE CONSTITUENTS OF THE STEM-XYLEM OF *HERNANDIA OVIGERA* L. (II)

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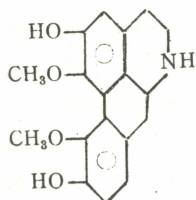
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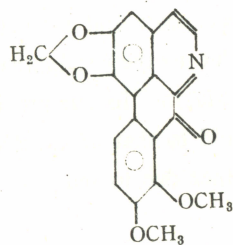
As in our previous report⁽¹⁾, a significant cytotoxic principle, desoxy podophyllotoxin (I) and hernovine (II) had been isolated from the stem-xylem of *Hernandia ovigera* L., it was also described that a small amount of two unknown bases were coexisted in the same part of this plant. We now wish to report on the isolation and structural elucidation of these two unknown bases (C & D) along with the isolation and identification of ovigerine (VII) and hernangerine (VIII).



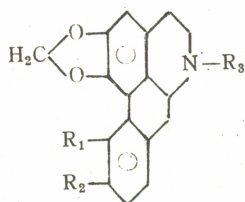
(I)



(II)



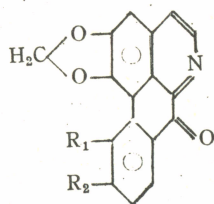
(III)



(IV) $R_1=R_2=OCH_3, R_3=CH_3$

(VI) $R_1, R_2 = \begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ \text{---} \end{array} \text{CH}_2, R_3=H$

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



(V) $R_1=R_2=OCH_3$

(VII) $R_1, R_2 = \begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ \text{---} \end{array} \text{CH}_2$

(IX) $R_1=R_2=H$

Compound-(C) was crystallized from ethanol as pale yellowish needle, mp. 222-224°. The mass spectrum showed the molecular ion peak at m/e 335 corresponding to the molecular formula $C_{19}H_{13}O_5N$. It gave positive Labat's test and formed a cherry red precipitates with diluted mineral acid. The infrared spectrum in chloroform at 1660 cm^{-1} indicated the presence of conjugated carbonyl group. The ultraviolet spectrum showed the maximum absorptions at 420 m μ ($\log \epsilon$ 4.16), 361 (4.17), 275 (4.41) and 225 (4.48) indicating a characteristic to the compound of an oxoaporphine alkaloid. The nmr spectrum in deuteriochloroform appeared two methoxyl groups as singlet at 6.12 τ , one methylenedioxy group as singlet at 3.69 τ , one

singlet aromatic proton at 2.17 τ attributed to C-3 aromatic proton and two pairs of adjacent aromatic protons centered at 1.68, 2.85 τ ($J=8.0$ Hz) and 1.07, 2.15 τ ($J=5.5$ Hz) respectively. All naturally occurring aporphine alkaloids which are known so far are substituted in positions 1 and 2, especially methylenedioxy group existed in the molecule. So the remained two oxygen function as the methoxyl groups of this compound must be attached at position 8 and 9 or 10 and 11 on the D ring, and thus the possible structure of compound-(C) must be either III or V. In order to determine the positions of these two methoxyl groups, oxidation of O, N-dimethylhernangerine (IV) with chromium trioxide-pyridine complex⁽²⁾ afforded V, as a yellowish needles, mp. 237-240° (CHCl₃-MeOH). It gave positive Labat's test. The infrared spectrum in KBr disc showed the absorption peaks at 1675 cm⁻¹ (conjugated carbonyl), 1590 (phenyl) and 1020, 960 (methylenedioxy). The ultraviolet spectrum showed the maximum absorptions at 409 m μ ($\log \epsilon$ 4.14), 356 (4.17), 310 sh (3.76), 290 sh (3.99) 269 (4.32) and 254 (4.41) indicating a characteristic to oxoaporphine alkaloid. The nmr in trifluoroacetic acid appeared two methoxyl groups at 6.28 τ and 6.31, one methylenedioxy group as singlet at 3.91 τ , one singlet aromatic proton at 2.89 τ and two pairs of adjacent aromatic protons centered at 1.92 τ , 2.99 ($J=9.0$ Hz) and 1.78, 2.07 ($J=6.0$ Hz), Which was found distinctly different with compound-(C) by their tlc, mixed melting point and ir comparison. Thus the structure V was eliminated by direct comarison as mentioned above, therefore the structure of compound-(C) is assigned to the formula III. It is unusual that the first known oxoaporphine bearing two methoxyl groups between C-8 and C-9, 1,2-methylenedioxy-7H-8, 9-dimethoxydibenzo [de, g] quinolin-7-one (III).

Compound-(D) was crystallized from chloroform as golden yellowish needles, mp. 287-290°. It gave positive Labat's test. The compound gave a red color in the mineral acid and its chloroform solution exhibited a green fluorescence. Its infrared spectrum (KBr) at 1650 cm⁻¹ indicated the presence of conjugated carbonyl group, but no NH or OH absorption. The ultraviolet spectrum maximum absorptions at 426 m μ , 365, 266 and 220 showed a characteristic to oxoaporphine chromophore as found in compound-(C). The nmr spectrum in trifluoroacetic acid of this compound showed signals due to two methylenedioxy groups at 4.07 and 3.87 τ , an unsplit aromatic proton at 2.85 τ and two pairs of adjacent aromatic protons centered at 1.98, 3.15 τ ($J=8.0$ Hz) and 1.68, 2.00 ($J=6.0$ Hz). These above data of compound-(D) were likewise in accord with published data of hernandonine (VII)^(3,4). Hernandonine (VII) is an oxidation product of ovigerine (VI), the cooccurrence of both ovigerine (VI) and hernandonine (VII) in the same plant is a biogenetic interesting fact.

Compound-(VI) was a alkali-insoluble base, the residue of nonphenolic fraction after removal of compound-(C) and (D) was rechromatographed on silica gel column, CHCl₃-MeOH (98:2) eluates was yielded a pale yellowish crystal, mp. 241-244° (MeOH), crystallized from acetone, mp. 122-124° (polymorphism). It gave

positive Labat's test. The ultraviolet spectrum showed the maximum absorptions at 312 m μ , 278, 269 and 235, and its tlc, mixed melting point and ir comparison with that of the authentic sample of ovigerine (VI)^(5,6) were found identical.

Compound-(VIII) from the mother liquid of alkali-soluble base after removal of hernovine (II) was crystallized as hydrobromide, mp. 222-225° (EtOH). It revealed positive to Labat's test and negative to Gibbs reaction. The infrared spectrum absorption peaks at 3500 cm⁻¹ indicated the phenolic hydroxyl group, 1590 cm⁻¹ was phenyl group and 1060, 950 cm⁻¹ showed the methylenedioxy group. The ultraviolet spectrum showed the maximum absorptions at 308 m μ , 271 and 227 indicating a characteristic to 1, 2, 10, 11-tetrasubstituted aporphine base⁽⁷⁾. This base was identified as hernangerine (VIII)^(7,8) by their tlc, mixed melting point and ir comparison.

In this time we have isolated two oxoaporphine alkaloids, a new base 1,2-methylenedioxy-7H-8,9-dimethoxydibenzo- [de, g] quinolin-7-one (III) and hernandoline (VII) from the stem-xylem of *Hernandia ovigera* L. Although it still waits further study to ascertain the action of these bases, but their structures are similar to oxoushinsunine (IX), as they both contained α, β -unsaturated ketonic moiety, which is an important group for the maintenance of high level cytotoxicity in sesquiterpenoids⁽⁹⁾. It is worthy to note that oxoushinsunine (IX) showed a significant tumor inhibitory activity against human carcinoma of the nasopharynx reported recently by David Warthen *et al.*⁽¹⁰⁾.

EXPERIMENTAL

All melting points were measured by use of the Yanagimoto Micromelting Point Apparatus and uncorrected. The infrared spectra were recorded with Hitachi Grating Infrared Spectrophotometer, model EPI-G2. The Ultraviolet spectra were taken on the Hitachi Double Beam 124 Spectrophotometer. The nmr spectra were obtained in scale with TMS as external reference, in deuterchloroform and trifluoroacetic acid. The mass spectrum was recorded on Hitachi model RMS-4 using a direct inlet system at an ionization energy at 70 eV.

Isolation of alkaloids:

As described in our previous report⁽¹⁾ the isolation of the constituents from the stem-xylem of *Hernandia ovigera* L. was firstly macerated with n-hexane, and then extracted successively with hot ethanol. The ethanolic extract after acid-base treatment was separated into alkali soluble and alkali insoluble bases. The alkali insoluble base (15 g.) was chromatographed on alumina column (4 \times 28 cm, Wako active alumina, 300 mesh), and continuously eluted with n-hexane, n-hexane-CHCl₃ (1:1), CHCl₃, CHCl₃-MeOH (20:1), CHCl₃-MeOH (1:1) and MeOH. The n-hexane-CHCl₃ (1:1) eluates yielded compound-(C) (30 mg), mp. 222-224° (EtOH). The CHCl₃ and CHCl₃-MeOH (20:1) eluates afforded compound-(D) (104 mg), mp. 287-290° (CHCl₃). The other fractions combined was rechromatographed on alumina column, eluted with CHCl₃ to give a small amount of compound-(C) (20 mg), and CHCl₃-MeOH

(98:2) eluates to yield compound-(VI) (142 mg) mp. 241-244° (MeOH), 122-124° (acetone, (polymorphism)). The mother liquid (5.5 g) after removal of hernovine (II) was dissolved in a small amount of acetone and 38% hydrobromic acid was added until acidic, cooled and stood overnight to give crystalline solid (1.42 g.). Repeated crystallization from ethanol afforded compound-(VIII) hydrobromide, mp. 222-225° (decomp.).

1, 2-methylenedioxy-7H-8, 9-dimethoxydibenzo [de, g] quinolin-7-one (III):

Pale yellowish needles, mp. 222-224° (EtOH), Mass spectrum: M^+ m/e 335 ($C_{19}H_{13}O_5N$). Labat's test: (+). It formed a cherry red precipitates in diluted mineral acid. ir ($CHCl_3$) cm^{-1} : 1660 (conjugated carbonyl). uv λ_{max}^{EtOH} μ ($\log \epsilon$): 420 (4.16), 361 (4.17), 275 (4.41) and 225 (4.48), nmr ($CDCl_3$) τ : 6.12 (6H, s, $2 \times OCH_3$), 3.69 (2H, s, $\begin{array}{c} \text{---O} \\ \diagup \quad \diagdown \\ \text{---O} \end{array} > CH_2$), 2.71 (1H, s, C-3aromatic proton), 1.68, 2.85 (2H, AB-quartet aromatic protons, $J=8.0$ Hz) and 1.07, 2.15 (2H, AB-quartet aromatic protons, $J=5.5$ Hz).

1, 2-methylenedioxy-7H-10, 11-dimethoxy dibenzo [de, g] quinolin-7-one (V), Chromium Trioxide-Pyridine complex Oxidation of O, N-dimethylhernangerine (IV):

A solution of O, N-dimethylhernangerine (IV) 215 mg (isolated from *Lindera oldhami*) in pyridine 15 ml was treated with chromium trioxide 1.5 g. in pyridine 9 ml, stirred in the cold for 8 hrs and stood 40 hrs at room temperature. Ethanol 10 ml followed by 200 ml of water was added and the solution was extracted thoroughly with $CHCl_3$. The combined $CHCl_3$ extracts were dried over anhydrous $MgSO_4$ and evaporated to leave a yellowish residue. Purification of this residue was chromatographed on SiO_2 -column, and eluted with $CHCl_3$ -MeOH (50:2). The residue from the eluates was crystallized from $CHCl_3$ -MeOH (10:1) to give a golden yellowish needles, V, 41 mg, mp. 237-240°. Labat's test: (+). It formed a red solution in the mineral acid. ir (KBr) cm^{-1} : 1675 (conjugated carbonyl), 1590 (phenyl) and 1020, 960 (methylenedioxy). uv λ_{max}^{EtOH} μ ($\log \epsilon$): 409 (4.14), 356 (4.17), 310sh (3.76), 290 sh (3.99), 269 (4.32) and 254 (4.41). nmr (F_3CCOOH) τ : 6.28, 6.31 (6H, $2 \times OCH_3$), 3.91 (2H, s, $\begin{array}{c} \text{---O} \\ \diagup \quad \diagdown \\ \text{---O} \end{array} > CH_2$), 2.89 (1H, s, aromatic proton), 1.92, 2.99 (2H, AB-quartet aromatic protons, $J=9.0$ Hz) and 1.78, 2.07 (2H, AB-quartet aromatic protons, $J=6.0$ Hz). Comparison III with V of their tlc, mixed melting point and ir were found distinctly different.

Hernandonine (VII):

Golden yellowish needles, mp. 287-290° ($CHCl_3$). Labat's test: (+). It formed a red color in the mineral acid and its chloroform solution exhibited green fluorescence under UV radiation. ir (KBr) cm^{-1} : 1650 (conjugated carbonyl), 1600 (phenyl), uv λ_{max}^{EtOH} μ ($\log \epsilon$): 426 (4.32), 365 (4.36), 266 (4.52) and 220 (4.58); nmr (F_3CCOOH)

τ : 3.87, 4.07 (4H, $2 \times \begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ \text{---} \end{array} \text{CH}_2$), 2.85 (1H, s, C-3 aromatic proton), 1.68, 2.00 (2H, AB-quartet aromatic protons, $J=6.0$ Hz) and 1.95, 3.15 (2H, AB-quartet aromatic protons, $J=8.0$ Hz). These above data of compound-(D) were likewise in accord with published data of hernandonine (VII)^(3,4).

Ovigerine (VI):

Pale yellowish rod-like crystal, mp. 122-124° (acetone), 241-244° (MeOH, polymorphism). Labat's tests: (+). ir (CHCl₃) cm⁻¹: 1600 (phenyl), 1070, 960. (methylenedioxy). uv $\lambda_{\text{max}}^{\text{EtOH}}$ μ (log ϵ): 312 (4.23), 278 (4.41), 269 (4.42) and 235 (4.42). It showed identical to ovigerine (VI) by direct comparison to tlc, mixed melting point and ir with an authentic sample.

Hernangerine (VIII):

Free base was difficultly crystallized, its hydrobromide, mp. 222-225° (decomp.) (EtOH). Labat's test: (+), Gibbs reaction: (-). ir (CHCl₃): 3500 (-OH), 1600 (phenyl) and 1060, 950 (methylenedioxy). uv $\lambda_{\text{max}}^{\text{EtOH}}$ μ (log ϵ): 308 (4.17), 271 (4.41) and 227 (4.52). Comparison of their tlc, mixed melting point and ir with authentic sample of hernangerine (VIII)-HBr were found identical.

SUMMARY

As in our previous report⁽¹⁾, a significant cytotoxic principle, desoxypodophyllotoxin (I) and hernovine (II) has been isolated from the stem-xylem of *Hernandia ovigera* L. Further investigation led to isolate a new base, 1,2-methylenedioxy-7H-8,9-dimethoxydibenzo [de, g] quinolin-7-one (III), hernandonine (VII), ovigerine (VI) and hernangerine (VIII). The structure III of this new alkaloid, mp. 222-224° (EtOH), C₁₉H₁₃O₅N, was established by the spectroscopic and chemical correlation with O,N-dimethylhernangerine (IV). III and VII are similar to oxoushinsunine (IX), as they both contained α, β -unsaturated ketonic moiety, which is an important group for the maintenance of high level cytotoxicity in sesquiterpenoids⁽⁹⁾. It is worthy to note that oxoushinsunine (IX) shows a significant tumor inhibitory activity against human carcinoma of nasopharynx reported recently by David *et al.*⁽¹⁰⁾.

ACKNOWLEDGEMENT

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- (1) T. H. YANG, S. T. LU and S. C. LIU.: Presented at the Annual Meeting of Taiwan Pharmaceutical Association, in Dec. 1971, and will be published elsewhere soon.
- (2) T. Y. YANG.: J. Pharm. Soc., Japan, **86**, 789-803 (1962)
- (3) K. ITO and H. FURUKAWA.: Tetrahedron Letters, **34**, 3023-3024 (1970)
- (4) M. P. CAVA and A. VENKATESWARLU.: Tetrahedron, **27**, 2639-2643 (1970)
- (5) M. P. CAVA, K. BESSHO, B. DOUGLAS, S. MARKEY, R. F. RAFFAUF and J. A. WEISBACH.: Tetrahedron Letters, **15**, 1577-1581 (1966)
- (6) M. TOMITA, S. T. LU and Y. Y. CHEN.: J. Pharm. Soc., Japan, **86**, 763-765 (1966)
- (7) MANSKE.: The Alkaloids, IX, p. 28 (1967)
- (8) H. FURUKAWA, S. T. LU.: J. Pharm. Soc., Japan, **86**, 1143-1147 (1966)
- (9) K. H. LEE, R. MECK, C. PIANTADOSI and E. S. HUANG.: J. Med. Chem., **16**, 299 (1973)
- (10) D. WARTHEN, E. L. GOODEN and M. JACOBSON.: J. Pharm. Sci., **58**, 627 (1969)

蓮葉桐 (*Hernandia ovigera* L.) 之 成分研究 (II)

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中 文 摘 要

前報⁽¹⁾自臺灣產蓮葉桐之心材單離一種對鼻咽癌有效成分 desoxypodophyllotoxin (I) 及一種第二級鹽基 hernovine (II)。今次再繼續其成分的檢討，結果又分離得 hernandonine (VII)，ovigerine (VI)，hernangerine (VIII) 及一新鹽基，mp. 222-224°，C₁₉H₁₃O₅N，此鹽基構造式之決定依其理化諸性質及光譜儀測定之數據及自 O, N-dimethylhernangerine (IV) 經 chromium trioxide-pyridine 氧化合成得之 1,2-methylenedioxy-7H-10, 11-dimethoxydibenzo [de, g]-quinolin-7-one (V)，mp. 237-240° 比較而推定為 1,2-methylenedioxy-7H-8,9-dimethoxydibenzo [de, g]-quinolin-7-one (III)。III 及 VII 之構造類似 oxoushinsunine (IX)，這些分子中均具有 α, β -unsaturated ketonic moiety，此基羣為 sesquiterpenoids⁽⁹⁾ 中發現抗癌作用之重要部分。又最近 David Warthen 等⁽¹⁰⁾報告 Oxoushinsunine (IX) 對人類鼻咽癌有效，故其抗癌作用似乎有關連。