

Studies on the Alkaloids of *Anona glabra* L. (II)

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During our investigations of Formosan Anonaceae, two alkaloids, (-) -N-methylactinodaphnine (I) and reticuline (II) were isolated from the leaves of *Anona glabra* L. and reported in the previous paper¹⁾. Now we wish to describe the continuation of our search for alkaloids from the stem wood and bark of this plant in this paper. As method described in the experimental part, the n-hexane and ethanol extract were examined leading to the isolation of one diterpenoid and ten alkaloids.

One yellowish needle crystal of Base-III. mp. 278—280° (decomp.) (CHCl_3) was obtained from the acidic ether and chloroform extract and is optical inactive. The presence of conjugated carbonyl absorption at 1650cm^{-1} in its IR spectrum and the formation of a deep cherry red solution with a diluted mineral acid. This base shows UV absorption maximum at $249\text{m}\mu$ ($\log \epsilon$ 4.37), 271 (4.34), 310 (4.08) and it also has absorption in the visible region. On the basis of these data, Base-III was assumed to be a oxoaporphine and identified as oxoushinsunine²⁾ (III) with an authentic sample by comparison of their IR (KBr), tlc and mixed melting point. Base-IV was characterized as a sparingly soluble hydrochloride, mp. 262—265° (decomp.) (EtOH), $[\alpha]_D^{24} = -53.2^\circ$ ($c=1$, EtOH). Its IR and UV spectral data indicates the identity with those of anonaine³⁾ (IV) hydrochloride.

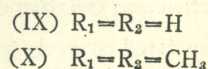
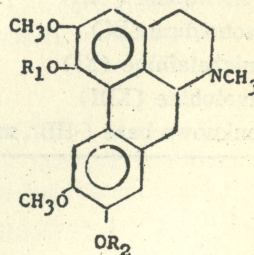
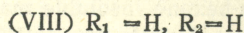
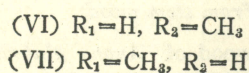
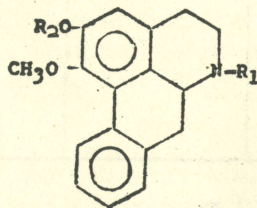
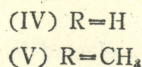
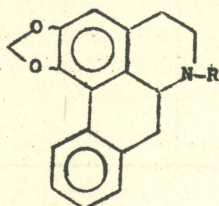
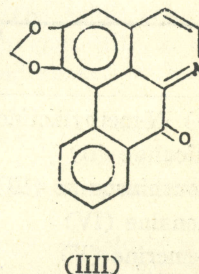
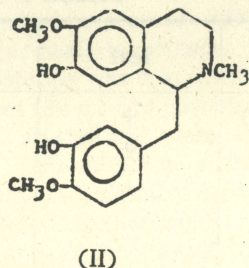
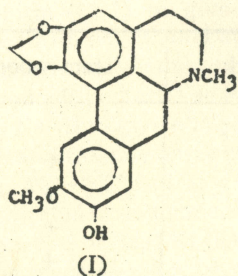
Base-V and VI, two oily alkali-insoluble alkaloids were isolated as crystalline hydrochlorides from the filtrate of anonaine (IV). Base-V (hydrochloride, mp. 260° (decomp.), $[\alpha]_D^{28} = -54^\circ$ ($c=0.67$, MeOH)) is identical to methylanonaine (roemerine)⁴⁾ (V), prepared from anonaine (IV) under methylation with formalin and formic acid by direct comparison of their IR spectra in Nujol mull and mixed melting point. The free base of Base-VI is an oily liquid and its hydrochloride, colorless needles, melts at 156—157° (EtOH), $[\alpha]_D^{27} = -144^\circ$ ($c=0.5$, EtOH). Its UV maximum absorption at $311\text{ m}\mu$ ($\log \epsilon$ 3.67), 271 (4.34), 230 (4.37) and 218 (4.42) reveal that it is a 1,2-disubstituted aporphine. The NMR signal exhibited two methoxyl at 6.30 and 6.10 τ , one down field aromatic proton as singlet at 1.60 τ assigned to C-11, one high field proton at 3.32 τ attributed to C-3 and the other three aromatic protons as doublet at 2.70 τ . Methylation with formalin and formic acid gave a crystalline product, mp. 163—164°, identical to authentic nuciferine⁵⁾ (VII). Therefore, Base-VI was proved to be N-nornuciferine⁶⁾ (VI).

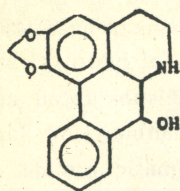
Base-II and VIII were characterized as crystalline perchlorate from phenolic fraction. The free Base-II, a colorless oil, gave deep blue color with Gibb's reagent and its perchlorate (mp. 203—205°) (EtOH), $[\alpha]_D^{28} = +85.3^\circ$ ($c=0.5$, EtOH), showed a maximum UV absorption at $285\text{ m}\mu$ ($\log \epsilon$ 4.11) and minimum absorption at $255\text{ m}\mu$ (3.23). Base-II was identical in all respects (IR, UV, tlc and Mixture melting point) with a sample of reticuline⁷⁾ (II) as perchlorate. The free base of Base-VIII was crystallized as colorless prisms from

acetone, mp. 172–174°, $[\alpha]_D^{28} = -213.4^\circ$ ($c=0.56$, CHCl_3). It showed UV maximum absorption at 307 $m\mu$ ($\log \epsilon$ 3.87), 273 (4.38), 210 (4.48) in ethanol and the IR bands of hydroxyl and secondary amino group at 3500 and 3250 cm^{-1} indicating a phenolic 1, 2-disubstituted and C-11 free occupied noraporphine⁶⁾. The NMR spectrum appeared one methoxyl at high chemical shift of 6.38 τ assigned to C-1 methoxy and other five aromatic protons at 1.66, 2.70 and 3.25 τ . The IR of N-nornuciferine (VI) in chloroform was superimposable with the O-methyl compound obtained by treating Base-VIII with diazomethane. The spectral data (UV, IR and NMR) of Base-VIII were likewise in accord with published data of asimilobine⁶⁾ (VIII) from *Asimina triloba* L.

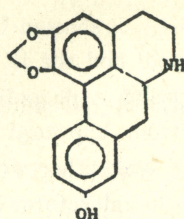
From the mother liquid of these two perchlorates, a phenolic Base-IX was also crystallized as hydrochloride, mp. 250°. Its UV maximum absorption at 306 $m\mu$ ($\log \epsilon$ 4.29), 282 (4.25), 220 (4.49) is very similar to a pattern of 1, 2, 9, 10-tetrasubstituted aporphine like glaucine (X)⁶⁾. The NMR signal of this base revealed two methoxyl as singlet at 6.08 τ , one N-methyl at 7.54 τ and three aromatic protons at 1.95, 3.20, 3.43 τ . O-methylation with diazomethane generated the same compound described for glaucine (X)⁷⁾. Therefore, this base proved to be identical with isoboldine⁸⁾ (IX). The isolated sample melted at 121–122°; reported melting point of isoboldine (IX) is 121–126°⁸⁾. Base-XI, mp. 204–206° together with anonaine (IV) was isolated as hydrochloride from alkaline ether extracted fraction and identified as nichelalbine⁹⁾ (XI) by comparison of UV, IR and mixed melting point.

The above eight alkaloids were obtained from the stem wood part. In addition, we also isolated oxoushinsunine (III), anonaine (IV), reticuline (II), michelalbine (XI) and two phenolic base (XII and XIII) from the ethanol extract of stem bark part of this same plant.

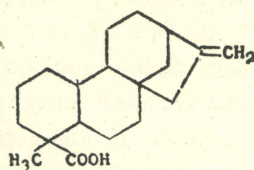




(XI)



(XIII)



(XIV)

The free base of XIII is pale yellow crystals from acetone and methanol, mp. 238–240° $[\alpha]_D^{25} = -21^\circ$ ($c=0.5$, MeOH). The UV absorption maximum at 282 m μ ($\log \epsilon$ 4.22), 220 (4.27) in ethanol and IR band at 3240, 930 and 1040cm $^{-1}$ showed that it is a 1, 2, 9-trisubstituted phenolic aporphine base⁹⁾. Base-XIII is identical to authentic anolobine²⁾ (XIII) showed by direct comparison of IR, tlc and admixture melting point. The other phenolic base-XII was characterized as crystalline hydrobromide, mp. 215–218° (EtOH). Identification of this alkaloid is now in progress.

The n-hexane extract of stem bark also afforded a crystalline compound, mp. 175–176° and it was identified as a plant regulator, (–)-kaur-16-en-19-oic acid¹⁰⁾ (XIV), previously isolated from *A. squamosa*. The identity was carried out by direct comparison with an authentic sample. After completion of our studies, a report appeared describing the isolation of oxoushinsunine (liriodenine) (III), a cytotoxic alkaloid against nasopharynx carcinoma, from stem wood and bark of Florida *Anona glabra* L.¹¹⁾. Besides, liriodenine (III), other nine alkaloids (II, IV, V, VI, VIII, IX, XI, XII, XIII) and one acid terpene (XIV) were also obtained from the same plant in our research work. The alkaloids of *Anona glabra* from its leaf, stem bark and wood are summarized in Table I.

TABLE I

	leaf	stem bark	stem wood
(–)-N-methylactinodaphnine (I)	+		
reticuline (II)	+	+	+
oxoushinsunine (III)		+	+
anonaine (IV)		+	+
roemerine (V)			+
N-nornuciferine (IV)			+
asimilobine (VIII)			+
isoboldine (IX)			+
michelalbine (XI)		+	+
anolobine (XIII)		+	
unknown base (-HBr, mp. 215–8°)		+	

Experimental

Extraction and isolation of alkaloids from stem wood:

The dried coarsely ground stem wood of *Anona glabra* L. (6.0kg.), collected in Taipei, was macerated with n-hexane. The n-hexane insoluble marc was refluxed with hot ethanol and the alcoholic extract was triturated with 3% AcOH. The acid soluble filtrate was

extracted with ether and then with CHCl_3 . After treatment with acid and base, each extract was evaporated to give ether soluble alkaloids (Fraction A, 3.7 g.) and chloroform soluble alkaloids (Fraction B, 3.3 g.). The acidic layer was made basic with conc.- NH_4OH and shaken with ether. The ether extractive was fractionated into base-insoluble alkaloids (Fraction D, 1.3 g.) and base-soluble alkaloids (Fraction C, 4.0 g.) by distributing between ether solution and 3% aq. NaOH ; the phenolic base were recovered from the latter by saturating it with solid NH_4Cl and then extracting with ether.

The ether extract of Fraction A was triturated and crystallized from chloroform to afford a yellow microneedle of Base-III, mp. 278–280° (decomp.) (0.1 g.). Fraction B was dissolved in CHCl_3 and evaporated to leave an orange solid which was recrystallized with same solvent as yellow needles of Base-III (0.2 g.). The mother liquid of III, was dissolved in ethanol and treated with 8% HCl - EtOH solution. The precipitated hydrochloride was collected and crystallized with ethanol to give Base-IV hydrochloride (0.6 g.), mp. 262–265° (decomp.). The filtrate of IV, treated with acid and base, was chromatographed on alumina column, eluted with chloroform to give two bases as pale yellow oil and converted to hydrochlorides of Base-V (5 mg.), mp. 260° (decomp.) (EtOH) and Base-VI (15 mg.), mp. 156–157° (EtOH).

The ethanol solution of phenolic Fraction C is treated with perchloric acid until acidic and evaporated off solvent with azeotropic distillation. Crystallization of residue with ethanol provided a colorless prism of Base-II perchlorate (0.5 g.), mp. 203–5°. Removing the most of ethanol from the filtrate of II gave another perchlorate of Base-VIII (0.6 g.), mp. 127–130°. The mother filtrate of this perchlorate was converted into hydrochloride salt to yield Base-IX- HCl (0.15 g.), mp. 250° (decomp.). The crude bases of Fraction D was transformed into their hydrochlorides and was found to be a mixture of two alkaloids in tlc-chromatogram. Fractional crystallization with ethanol afforded two crystals, one is Base-IV (-HCl , mp. 262–265°) (0.1 g) and the other is Base-XI (-HCl , mp. 260°) (60 mg.).

Extraction and isolation of alkaloids from stem bark:

The chopped stem bark (2.2 kg.) was extracted with cold n-hexane 3 times. The total n-hexane extract was subjected to silicic acid column chromatography, eluted with n-hexane-chloroform (2: 1) to give crystal XIV, mp. 175–176°. The n-hexane insoluble marc was refluxed with hot ethanol and the extracts was treated with 5% AcOH . The acidic solution was shaken with ether to remove non-basic substance, basified with conc.- NH_4OH and extracted with ether. The total ether solution was concentrated to 1.5 l and shaken with 3% aqueous NaOH to give non-phenolic bases (2.4 g.) and phenolic bases (4.1 g.).

Alkali-insoluble bases provided two crystalline bases as hydrochlorides of Base-IV (1.2 g.), mp. 262–265° and Base-XI (60 mg.), mp. 260°. The filtrate of these two hydrochlorides was subjected to column chromatography on alumina, eluted with chloroform to give yellow microneedles of Base-III (9 mg.), mp. 278–280° (decomp.).

The crude phenolic bases were dissolved in ethanol and treated with perchloric acid. After crystallization with ethanol colorless prisms of Base-II- HClO_4 (0.7 g.), mp. 203–205° were obtained. The free base from the filtrate of II was chromatographed on silicic acid column. Elution with 5% MeOH in CHCl_3 gave Base-XII as crystalline hydrobromide (30 mg.), mp. 215–218° and Base-II (0.1 g.). Continued elution with 10% MeOH in CHCl_3 yielded Base-XIII (5 mg.), pale yellow crystals, mp. 238–240° (decomp.).

Oxoushinsunine (liriodenine) (III):

Yellow microneedles, mp. 278–280° (decomp.) (CHCl_3), optically inactive. It formed a cherry red solution with mineral acids and exhibited a bright yellow fluorescence under uv radiation, IR(KBr) cm^{-1} : 1650 (conjugated C=O), 960, 1050 ($\text{—O—CH}_2\text{—O—}$). UV $\lambda_{\text{max}}^{\text{EtOH}}$ μm (log ϵ): 249 (4.37), 271 (4.31), 310 (4.08). UV $\lambda_{\text{min}}^{\text{EtOH}}$ μm (log ϵ): 264 (4.29), 293 (4.04), 341 (3.82). It is identical to oxoushinsunine by direct comparison with an authentic sample. Anal. calcd. for $\text{C}_{17}\text{H}_9\text{O}_4\text{N}$: C, 74.18; H, 3.30; N, 5.09 Found: C, 74.30; H, 3.21; N, 4.92.

Anonaine (IV):

Free base: colorless oil. Hydrochloride: colorless needle, mp. 262–265° (decomp.) (EtOH), $[\alpha]_D^{24} = -53.2^\circ$ (C=1, EtOH), Positive to Labat's test; UV $\lambda_{\max}^{\text{EtOH}}$ $m\mu$ (log ϵ): 320 (3.80), 274 (4.34), 216 (4.39), IR (nujol) cm^{-1} : 940, 1050 (—O—CH₂—O—). It is identical with an authentic sample of anonaine by direct comparisons (IR, tlc and mixed melting point).

Roemerine (V):

Free base: pale yellow oil, IR (CHCl₃): 940, 1050 cm^{-1} (—O—CH₂—O—), Positive to Labat's test. Its hydrochloride is a white crystal, mp. 260° (decomp.) (EtOH); $[\alpha]_D^{28} = -54^\circ$ (C=0.67, MeOH). This base was identical to N-methylanonaine (roemerine), obtained by treating anonaine with formic acid and formalin.

N-nornuciferine (VI):

Free base: an oily liquid. Hydrochloride: colorless crystal, mp. 156–157° (EtOH); $[\alpha]_D^{27} = -144^\circ$ (C=0.5, EtOH); UV $\lambda_{\max}^{\text{EtOH}}$ $m\mu$ (log ϵ): 218 (4.42), 230 (4.37), 271 (4.34), 311 (sh.) (3.67); NMR (CDCl₃) τ : 6.30, 6.10 (6H, two OCH₃), 3.32, 1.60 (2H, aromatic -H), 2.70 (3H, aromatic -H). Its N-methyl compound (VIII), mp. 163–164° (EtOH), $[\alpha]_D^{27} = -145^\circ$ (C=0.67, EtOH), was found identical to an authentic sample of nuciferine (VII).

Reticuline (II):

It is a Colorless liquid, positive to ferric chloride and Gibb's test; IR (CHCl₃): 3500 cm^{-1} (-OH). Its Perchlorate is a colorless prism, mp. 203–205° (EtOH), $[\alpha]_D^{28} = +85.30^\circ$ (C=0.5, EtOH); UV $\lambda_{\max}^{\text{EtOH}}$ $m\mu$ (log ϵ): 285 (4.11), 209 (4.59); UV $\lambda_{\min}^{\text{EtOH}}$ $m\mu$ (log ϵ): 255 (3.23). Anal. calcd. for C₁₉H₂₃O₄N·HClO₄·H₂O: C, 51.05; H, 5.86; N, 3.14. Found: C, 51.42; H, 5.98; N, 3.20. It was identical in all respects with an authentic specimen of reticuline (II).

Asimilobine (VIII):

Colorless prisms, mp. 172–174° (Me₂CO), $[\alpha]_D^{28} = -213.4^\circ$ (C=0.56, CHCl₃), IR cm^{-1} : 3250 (>NH) (in nujol), 3500 (-OH) (in CHCl₃); NMR (CDCl₃) τ : 6.38 (3H, OCH₃), 3.25, 1.66 (2H, aromatic -H), 2.70 (3H, aromatic -H); Hydrochloride: White crystal, mp. 226° (decomp.) (EtOH); UV $\lambda_{\max}^{\text{EtOH}}$ $m\mu$ (log ϵ): 307 (3.87), 273 (4.38), 230 (4.40), 210 (4.48). Perchlorate: colorless crystal, mp. 127–130° (EtOH). The hydrochloride of O-methyl derivative of VIII is a colorless crystal, mp. 156–157° (EtOH), and was found identical to the hydrochloride of N-nornuciferine (VI).

Isoboldine (IX):

White crystal, mp. 121–122° (EtOH), negative to Labat's and Gibb's test; $[\alpha]_D^{28} = +56^\circ$ (C=0.43, CHCl₃); IR (nujol) cm^{-1} : 3500 (OH); NMR (CDCl₃) τ : 7.54 (3H, one NCH₃), 6.08 (6H, two OCH₃), 3.20, 1.95 (2H, aromatic -H), 3.43 (1H, aromatic -H). Hydrochloride of IX, mp. 250° (MeOH); UV $\lambda_{\max}^{\text{EtOH}}$ $m\mu$ (log ϵ): 306 (4.29), 282 (4.25), 220 (4.49). The hydrobromide of 0, 0-dimethyl derivative of IX, mp. 215–216° (decomp.) (EtOH), was found identical to the hydrobromide of glaucine (X).

Michelalbine (XI):

Colorless prism, mp. 204—206° (MeOH), $[\alpha]_D^{26} = -103.7^\circ$ (C=0.5, CHCl₃); Positive to Labat's test; UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (log ϵ): 321 (3.64), 215 (4.22); IR (CHCl₃) cm⁻¹: 3240 (-OH) 1050, 940 (-O-CH₂-O-). Anal. calcd. for C₁₇H₁₅O₃N: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.71; H, 5.40; N, 4.89. Hydrochloride of XI Colorless needles, mp. 260° (decomp.) (EtOH), identical to the hydrochloride of michelalbine (XI).

Unknown base (XII):

The free base is an oily liquid. Its hydrobromide melts at 215—218° (decomp.) (EtOH).

Anolobine (XIII):

Pale yellow crystals, mp. 238—240° (MeOH and Me₂CO) (decomp.); $[\alpha]_D^{25} = -21^\circ$ (C=0.5, MeOH); Positive to Labat's and ferric chloride test; UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (log ϵ): 282 (4.22), 220 (4.27); IR (nujol): 3240 (>NH), 930, 1040 cm⁻¹ (-O-CH₂-O-). Anal. calcd. for C₁₇H₁₅O₃N: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.57; H, 5.36; N, 5.08. Hydrochloride of XIII mp. 245—248° (decomp.) (EtOH); The IR of this base was found superimposable with that of an authentic sample of Anolobine (XIII).

(-)-Kaur-16-en-19-oic acid (XIV):

Colorless prisms, mp. 175—176°; $[\alpha]_D^{28} = 109^\circ$ (C=1, EtOH); IR (CHCl₃) cm⁻¹: 1685, 2650, 2750, 3500 (-COOH), 3060, 1650, 870 (=CH₂). Anal. calcd. for C₂₀H₃₀O₂: C, 79.42; H, 9.99. Found: C, 79.34; H, 10.25. This compound was identified as (-) -kaur-16-en-19-oic acid from *A. squamosa* by direct comparison of IR, tlc and mixed melting point.

Summary

Investigation of the stem wood and bark from *Anona glabra* L. (Anonaceae) led to the isolation of ten alkaloids and one terpenoid. These include oxoushinsunine (III), anonaine (IV), roemerine (V), michelalbine (XI), N-nornuciferine (VI), asimilobine (VIII), reticuline (II), anolobine (XIII), isoboldine (IX), (-) -kaur-16-en-19-oic acid (XIV) and one unknown base, (hydrobromide, mp. 215—218°) which is examined in progress.

The alkaloids of *Anana glabra* from its leaf, stem bark and wood are summerized in Table I.

Acknowledgement

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中 文 摘 要

圓滑蕃荔枝 (*Anona glabra* L.) 生物鹼之研究(II)

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臺 北 醫 學 院 藥 物 化 學 科

從抗癌性植物圓滑蕃荔枝 (*Anona glabra* L.) 之葉部曾分離一種新生物鹼 (—)-N-methylactinodaphnine 及 reticuline 等二種鹽基。今次繼續精查其幹皮及心材部成分結果得十種鹽基及一種 terpenoid，即 oxoushinsunine (III), anonaine (IV), roemerine (V), michelalbine (XI), N-nornuciferine (VI), asimilobine (VII), reticuline (II), anolobine (XIII), isoboldine (IX), (—)-kau-16-en-19-oic acid (XIV) 及一未知鹽基(HBr mp. 215~218°)，這些生物鹼存在本植物各部分之分佈狀況如表 I。