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Studies on the Constituents of *Anona squamosa* L.

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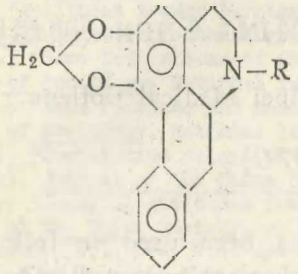
Anona squamosa L. (Fam. Anonaceae) has been used in folk medicine[1] as amebicide, tonic, astringent and has antitumor activity against Ehrlich cancer cell in mice[2,3]. In order to search for the active constituents of Formosan antitumor plants, examination was made on the alkaloids of the roots of *Anona squamosa* L.

Seven alkaloids (A-G) and an non-alkaloidal compound (H) were isolated from the basic and n-hexane fraction respectively. Identification of these bases by spectral analysis and direct comparison of their infrared spectra, tlc and mixed melting point have proved to be non-phenolic anonaine (I), michelalbine (III), oxoushinsunine (liriodenine) (IV) and phenolic L-(+)-reticuline (V), anolobine (VI). Examination of other two minor phenolic Base-D, mp. 224-226°, Base-E, mp. 231-233° (decomp) and an non-alkaloidal crystal-H, mp. 175-176° are now in progress.

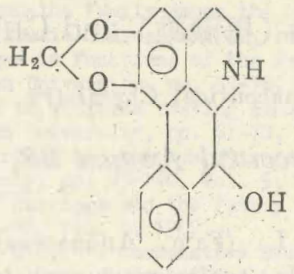
Anona squamosa L. (Fam. Anonaceae) is generally known as a tropical trees which is distributed around the south area of Taiwan. Its stem bark and fruit are used in folk medicine[1] as amebicide, tonic and astringent. K. Yamaguchi, *et al*[2,3] described that the dried fruit and leaves of this plant have antitumor activity against Ehrlich cancer cell in mice. On the constituents of *Anona squamosa L.*, it has been reported by Santos, *et al*[4,5] and Trimurti[6] that anonaine[7] (I) was isolated from the seeds and leaves. However, further investigations by thin-layer chromatography disclosed that it contained several other chemical constituents. In this paper, we wish to report the isolation and identification of alkaloids from the roots of *Anona squamosa L.*

The extraction and isolation of alkaloids from the roots was described in detail in the experimental part. There are seven alkaloids, Base-A, -B, -C and Base-D, -E, -F, -G which were isolated

from the non-phenolic and phenolic fraction of alcoholic extracts. In addition, an non-alkaloidal crystal-H, *mp.* 175-176° was also obtained from the n-hexane extracted portion.



(I) R=H (II) R=CH_3



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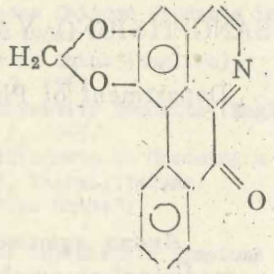
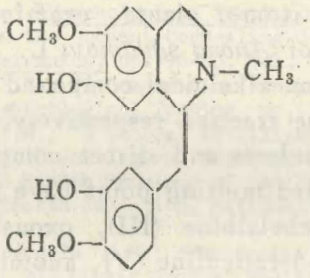
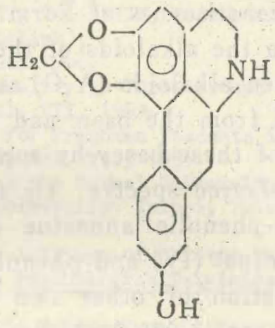


(IV)



(V)



(VI)

Base-A is an oily base which was crystallized as hydrochloride from ethyl alcohol, $C_{17}H_{15}O_2N \cdot HCl$, *mp.* 262-265° (decomp.), $[\alpha]_D^{24}$ -53.2° (c=1, EtOH). The UV spectrum of this base had maximum absorption at 270 $m\mu$ (log ε 3.94) and 320 $m\mu$ (log ε 3.28). It gave positive Labat's color reaction and the infrared bands at 940, 1050 and 2550, 2480 cm^{-1} indicating the presence of methylenedioxy and imino groups. The *N*-methyl derivative yielded upon methylation with formalin and formic acid as crystalline white scales, *mp.* 260° (decomp). Therefore, Base-A was assumed as anonaine[7] (I) and identified by IR(nujol), tlc and mixed melting point comparison with authentic sample and its *N*-methyl compound, roemerine (II).

The free base of Base-B is colorless prisms, *mp.* 204-206°, $[\alpha]_D^{26}$ -103.7° (c=0.5, $CHCl_3$), $C_{17}H_{15}O_3N$. It shows UV absorption maximum at 275 $m\mu$ (log ε 4.23) and 328 $m\mu$ (log ε 3.49) and the infrared bands at 940, 1050 and 3240 cm^{-1} indicating the presence of

methylenedioxy and alcoholic hydroxyl groups at aporphine nucleus. The NMR signals exhibited one proton doublet of the secondary alcoholic methine peak at 5.50 τ, two one proton doublets of methylenedioxy peak at 3.91 and 4.04 τ, one proton of imino or hydroxyl at 6.26 τ, one proton singlet of the C_3-aromatic proton at 3.36 τ, one proton multiplet corresponding to C_{11}-aromatic proton at 1.95 τ, and three protons multiplet of the $C_{8,9,10}$-aromatic proton at 2.50-2.75 τ. This base was identified with michelalbine[8] (III) as hydrochloride by mixed melting point, tlc and comparison of infrared spectra.

The minor non-phenolic Base-C was crystallized from mother liquid of Base-A and Base-B as yellowish microneedles, *mp.* 278-280° (decomp), $C_{17}H_9O_3N$, positive Labat's color test. The infrared spectrum at 1650 cm^{-1} showed conjugated carbonyl group and extremely low hydrogen to carbon composing ratio suggested Base-C is a highly unsaturated molecule, 7H-dibenzo [de, g]-quinolin-7-one series. Thereupon, this base was identified as oxoushinsunine (liriodenine)[9] (IV) by tlc, mixed melting point and infrared spectra comparison.

The free base of phenolic Base-F is a colorless oily liquid which was characterized as crystalline oxalate, *mp.* 154-155° and perchlorate, colorless cubics, *mp.* 203-205°, $[\alpha]_D^{28}$ +85.71° (c=0.54, EtOH), $C_{19}H_{23}O_4N \cdot HClO_4 \cdot H_2O$. The UV spectrum of this base was characteristic of benzyltetrahydroisoquinoline series giving the absorption maximum at 285 $m\mu$ and minimum at 255 $m\mu$. The nature of the hydroxyl group, revealed by the Gibbs' test, is phenolic with an unsubstituted para position. Consequently, Base-F was identified with L-(+)-reticuline[10] (V) as perchlorate by tlc, mixed melting point and infrared(nujol) comparison.

The minor, phenolic Base-G is isolated as hydrochloride, pale yellow needles, *mp.* 245-248° (decomp) (EtOH). The free base is pale yellow crystals from acetone and methanol, *mp.* 238-240° (decomp), $C_{17}H_{15}O_3N$, positive Labat's, ferric chloride and false Gibbs' reaction. It shows a molecular ion peak at *m/e* 281 in its mass spectrum

confirming the molecular formula to be $C_{17}H_{15}O_3N$ and the UV absorption maximum at 232 $m\mu$ (log ε 4.29) and 320 $m\mu$ (log ε 3.60) in ethanol solution. The IR bands at 3240, 1600, 930 and 1040 cm^{-1} indicated this base is a phenolic secondary base comprising methylenedioxy group, and its NMR signals exhibited two one proton doublet of the methylenedioxy peak at 3.91 and 4.05 τ. From these results, Base-G was considered to be anolobine[11] (VI) and identified by mixed melting point, tlc and comparison of infrared spectra.

The identification of the minor phenolic unkown Base-D, *mp.* 224-226°, Base-E, *mp.* 231-233° and non-alkaloidal crystal-H, *mp.* 175-176° is now under investigation.

It still waits further study to ascertain which isolated constituents represent it antitumor action of this plant. It is interesting that the co-occurence of these five related alkaloids, reticuline(V), anonaine(I), anolobine(VI), michelalbine(III) and oxoushinsunine (liriodenine)(IV) suggests a realistic sequence of biosynthetic transformations.

Experimental

All melting points are uncorrected and determinated with Yanagimoto micro melting point apparatus. The optical rotations were measured with Rex Photoelectric Polarimeter, model NEP-2. IR spectra were recorded with Hitachi Grating Infrared Spectrophotometer, model EPI-G2. The NMR signals were obtained in τ units using a Varian A-60A Spectrometer. The mass spetcrum was recorded on Hitachi model RMU-6A using a direct inlet system at an ionizing energy of 75 eV. Thin-layer chromatography was performed on silical gel F254 (E. Merck) with $CHCl_3$-MeOH(5-2) as developing solvent and detection was carried out by spraying Dragendorff's reagent.

Isolation of Alkaloids:

The roots (2.1 kg.) of *Anona squamosa L.*, collected in Tainan district, Formosa in February 1968, were macerated with *n*-hexane three times. Repeated recrystallization of the *n*-hexane extract

with methanol afforded the non-alkaloidal crystal-H, *mp* 175-176°. The marc of *n*-hexane extract was refluxed with hot EtOH until negative Mayer's test and the total alcohol extract was concentrated in vacuum to syrup. The residue(200 *g.*) was dissolved in 3% AcOH, filtered, and washed with Et_2O to remove the neutral and acidic substances. The acidic solution was made alkaline with *c*-NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ solution was shaken with 3% NaOH aq. solution to separate the phenolic and non-phenolic bases. The lower $CHCl_3$ layer was washed with water, dried over anhyd. K_2CO_3 and evaporated to leave a crude non-phenolic base (2.7 *g.*). The upper NaOH solution was made weak basic with excess NH_4Cl and extracted with $CHCl_3$. After washing with water and drying over anhyd. K_2CO_3, the $CHCl_3$ solution was concentrated to afford a crude phenolic base (6.6 *g.*). The water-soluble quaternary base was precipitated as base reineckate (22.5 *g.*) which is examinated in progress.

The crude non-phenolic base was dissolved in a small amount of EtOH and 8% HCl-EtOH solution was added until acidic to yield a crude crystalline hydrochloride (1.7 *g.*). Fractional crystallization with ethyl alcohol gives Base-A hydrochloride (0.8 *g.*), *mp.* 262-265° (decomp) and Base-B hydrochloride (50 *mg.*), *mp.* 260° (decomp). The mother liquid of this hydrochloride, after acid-alkali treatment, was crystallized from $CHCl_3$ to yielded yellowish microneedles, Bace-C (30 *mg.*), *mp.* 278-280° (decomp).

The crude phenolic base was chromatographed on SiO_2 (150 *g.*) (Wakogel, C-300) colum. Evaporation of the first two $Me_2CO-CHCl_3$ (1-1) eluted fractions and recrystallization with MeOH gives Base-D (20 *mg.*), *mp.* 224-226° and Base-E (40 *mg.*), *mp.* 231-233° (decomp). The third $Me_2CO-CHCl_3$ (1-1) eluted fraction afforded oily Base-F (0.2 *g.*) which characterized as crystalline perchlorate, mp. 203-205°. Continued elution with MeOH yielded Base-G (5 *mg.*) as hydrochloride, *mp.* 245-248° (decomp).

Base-A. anonaine (I):

Hydrochloride: colorless needles, *mp.* 262-265° (decomp), $[\alpha]_D^{24}$ −53.2° (c=1, EtOH), positive Labat's and negative ferric chloride test. UV $\lambda_{max}^{EtOH} m\mu$ (log ε): 270 (3.94), 320 (3.28). IR (nujol): 940, 1050 cm^{-1} (—O—CH_2—O—), 2500, 2550 cm^{-1} ($=\overset{+}{N}H_2$). Anal. calcd. for $C_{17}H_{15}O_2N \cdot HCl$. C, 67.66; H, 5.35; N, 4.64. Found: C, 67.87; H, 5.59; N, 4.35. N-methylation of I yielded II and characterized as hydrochloride, white scales, *mp.* 260° (decomp) (EtOH). This base and its N-methyl derivative hydrochloride were identical to IR(nujol), tlc and mixed melting point with anonaine(I) and roemerine(II) respectively.

Base-B. michelalbine (III):

Colorless prisms, *mp.* 204-206° (MeOH), $[\alpha]_D^{26}$-103.7° (c=0.5, $CHCl_3$) positive Labat's and negative ferric chloride reaction. UV $\lambda_{max}^{EtOH} m\mu$ (log ε): 275(4.23), 328(3.49). IR($CHCl_3$): 3240 cm^{-1} (-OH); 1050, 940 cm^{-1} (-O-CH_2-O-). NMR(d_6DMSO): 5.50 τ (1H, -CHOH, doublet, J=3 *cps*); 3.91, 4.04 τ (2H, -O-CH_2-O-, 2 doublets); 6.26 τ (1H, =NH or -OH); 6.50-7.60 τ (6H, multiplet); 3.36 τ (1H, C_3-H, singlet); 1.95 τ (1H, C_{11}-H, multiplet); 2.50-2.75 τ (3H, $C_{8,9,10}$ aromatic protons, multiplet). Anal. calcd. for $C_{17}H_{15}O_3N$. C, 72.58; H, 5.37; N, 4.98. Found: C, 72.71; H, 5.40; N, 4.89. Hydrochliride: colorless needles, mp. 260° (decomp)(EtOH). This base-HCl was identical to michelalbine (III)-HCl by IR(nujol), mixed melting point and tlc comparison.

Base-C. oxoushinsunine (liriodenine) (IV):

Yellowish microneedls from $CHCl_3$, mp. 278-280° (decomp), $[\alpha]_D^{30}$ ± 0° (c=0.5, C_5H_5N). IR(nujol): 1650 cm^{-1} (conjugated C=O); 960, 1050 cm^{-1} (-O-CH_2-O-). Bright yellow fluorescence under uv light. Anal. calcd. for $C_{17}H_9O_3N$. C, 74.18; H, 3.30; N, 5.09. Found: C, 74.30; H, 3.21; N, 4.92. Base-C was identified as oxoushinsunine (liriodenine) (IV) by IR(nujol), tlc and mixed melting point comparison

Base-D:

An unknown minor phenolic alkaloid, *mp.* 224-226° (MeOH).

Base-E:

An unknown minor phenolic alkaloid, *mp.* 231-233° (docomp) (MeOH).

Base-F. L-(+)-reticuline (V):

Colorless oily liquid, positive ferric chloride and Gibbs' tests, and negative Labat's test. UV λ_{max}^{EtOH}: 285 $m\mu$, λ_{min}^{EtOH}: 255 $m\mu$. IR($CHCl_3$): 3500 cm^{-1} (phenolic -OH). Oxolate: *mp.* 154-155° (Me_2CO+MeOH). Perchlorate: colorless cubics, *mp.* 203-205° (EtOH), $[\alpha]_D^{28}$+85.71° (c=0.54, EtOH). Anal. calcd. for $C_{19}H_{23}O_4N \cdot HClO_4 \cdot H_2O$. C, 51.05; H, 5.86; N, 3.14. Found: C, 51.42; H, 5.98; N, 3.20. This base perchlorate was identical as L-(+)-reticuline(V) perchlorate by IR(nujol), tlc and melting point comparison.

Base-G. anolobine (VI):

Pale yellow crystals, mp. 238-240° (decomp) (Me_2CO+MeOH), $[\alpha]_D^{22}$-21° (c=0.5, MeOH), positive Labat's and ferric chloride reactions and false Gibbs' reaction. UV λ_{max}^{EtOH} $m\mu$ (log ε): 282(4.29), 320(3.60). IR(nujol): 3240 cm^{-1} (=NH); 1600 cm^{-1} (phenyl); 930, 1040 cm^{-1} (-O-CH_2-O-). NMR($CDCl_3$): 3.91, 4.05 τ (2H, -O-CH_2-O-, 2 doublets). Mass spectrum: M^+ *m/e* 281 ($C_{17}H_{15}O_3N$); other intense peaks: *m/e* 280, 252, 222, 194, 165, 152 and 140. Anal calcd. for $C_{17}H_{15}O_3N$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.57; H, 5.36; N, 5.03. Hydrochloride, pale yellow needles, *mp.* 245-248° (decomp) (EtOH). The IR(nujol) spectra, tlc and mixed melting point of this base were identical with anolobine (VI).

Crystal-H:

An unknown non-alkaloidal compound, mp. 175-176° (MeOH).

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