CONSTITUENTS OF THE LEAVES OF *CISSUS RHEIFOLIA*[1]

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ABSTRACT.—The major alkaloid of the leaves of *Cissus rheifolia* Planch. (Vitaceae) has been identified as a new quinolizidine, which has been named kayawongine and has been assigned the structure *trans*-2-(3,4-dimethoxyphenyl)-3-(4-methoxyphenyl)quinolizidine (**2**) by analysis of the spectral data. Also identified in the plant were the alkaloid cryptopleurine (**1**), the isomeric terpenoids vomifoliol (**3**) and romalea allene (**4**), and the major flavonoid vitexin (**5**).

In the rural areas of northeast Thailand, the plant *Cissus rheifolia* Planch., or *Ka-ya-wong*, is reputed to have some medicinal value. The Thai name for the plant, which translates as "vulture without hope," derives from a local legend about the recovery of a herd of cattle from an unspecified disease after they were fed quantities of rice boiled with the roots of the plant. The waiting vultures thus lost all hope for an anticipated feast. Preliminary screening of the plant material showed a positive test for alkaloids. Because this plant belongs to the largest genus of the family Vitaceae, a family in which alkaloids have been reported but not structurally characterized, a full phytochemical investigation was initiated.

Defatted alcoholic extracts of the leaves of *C. rheifolia* were partitioned between chloroform and aqueous methanol. Processing of the chloroform extract by column chromatography gave fractions from which the known alkaloid cryptopleurine (**1**) and the new alkaloid kayawongine (**2**), as well as the terpenoids vomifoliol (**3**) and romalea allene (**4**), were obtained. The aqueous methanolic layer yielded a mixture of flavonoids from which vitexin (**5**) was isolated by recrystallization.

The minor alkaloid has been identified as **1** ($C_{24}H_{27}NO_3$) by comparison of its spectral properties with the reported ^{1}H-nmr spectrum (1) and mass spectrum (2) of cryptopleurine. Moreover, while working with fractions from which **1** was subsequently isolated, one of us (E.S.) experienced the vesicant action previously reported for cryptopleurine (3).

The optical purity of **1** from *C. rheifolia* is low, as evidenced by the decreased intensity of the short wavelength, positive absorption maximum in its circular dichroism (cd) spectrum (4). However, the position and the sign of the cd maximum indicate that the natural *R*-isomer (4) is present in excess. Partially racemized cryptopleurine has been previously isolated from plants of the genus *Boehmeria* (Urticaceae) (5,6).

The major alkaloid, **2**, named kayawongine after the Thai name of the plant, shows its molecular ion in the mass spectrum at *m/z* 381.228 indicating a composition of $C_{24}H_{31}NO_3$ (calculated 381.230). Thus, **2** contains four more hydrogen atoms than does **1**. The mass spectral fragmentation pattern (figure 1) indicates that the aromatic rings in **2** are not joined together in a phenanthrene nucleus as are the aromatic rings in **1**. The mass spectrum of **2** shows three major fragmentation pathways involving cleavage of the aryl-substituted ring of the quinolizidine skeleton, as depicted in figure 1. Cleavage at *a* gives rise to ions at *m/z* 270, 111, and 110. Cleavage at *b* gives ions at *m/z* 217, 216, and 164. Cleavage at *c* gives the base peak at *m/z* 134, apparently as the only charge-bearing fragment (no ions $>1\%$ in relative abundance are observed at *m/z* 245-

[1]Taken from the Ph.D. dissertation of Ekarin Saifah (1981); the spectral data reported in this paper are reproduced in the dissertation.

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247). Other significant ions in the spectrum at *m/z* 273 and 243 result from the loss of the equivalents of neutral anisole and veratrole.

Consideration of the mass spectral data allows the relative placement of the 4-methoxyphenyl substituent at C-3 rather than at C-2 of the quinolizidine ring. The proposed structures for the pair of ions observed at *m/z* 217 and 216 (see figure 1) each show the positive charge in the stabilized electronic configuration of an ammonium ion. Structures with ammonium ion stability cannot be drawn for ions with these masses if the placement of the aryl substituents is reversed. Additionally, ions 30 amu larger than the ions at *m/z* 217 and 216 are absent from the mass spectrum of **2**. The mass spectrum clearly shows that the new alkaloid has a placement of the aromatic rings similar to **1**, the structure of which has been verified by X-ray analysis (7).

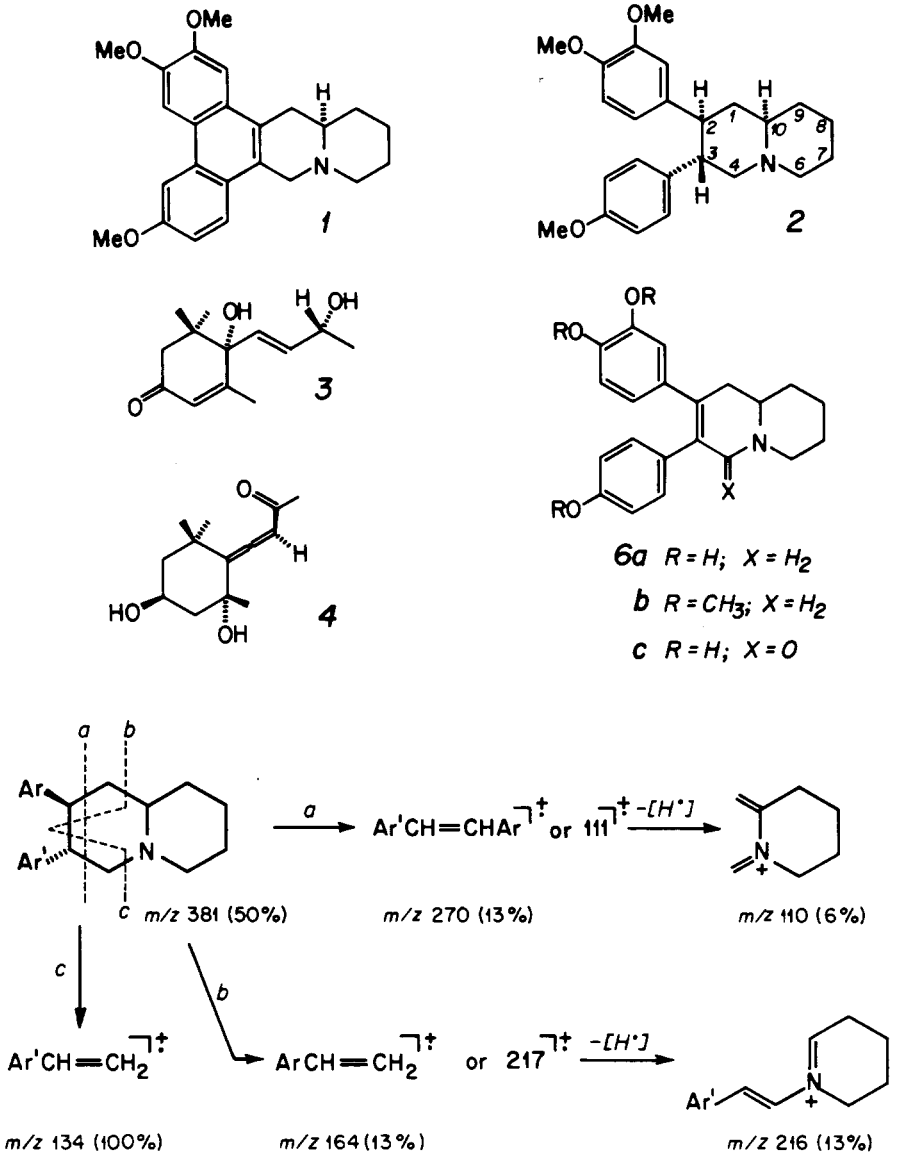

FIGURE 1. Kayawongine (**2**), ms fragmentation pattern.

The ^{1}H-nmr spectrum of **2** was determined at 270 MHz, and a series of decoupling experiments were carried out to identify the individual resonances. Of paramount interest is the identification of the resonances of H-2 and H-3, each occurring as a triplet (J=11 Hz) of doublets (J=4 Hz) at δ 2.76 and 3.10, respectively. Analysis of the coupling pattern shows that $J_{2,3}$=11 Hz, thus requiring H-2 to have a *trans*-diaxial relationship to H-3. The aryl groups then occupy equatorial positions on the quinolizidine ring. Also obtained from the ^{1}H-nmr analysis are the relative configurations for H-2 and H-10 by the identification of the resonance of H-1 (eq) as a doublet (J=11 Hz) of triplets (J=4 Hz) at δ 1.88. The presence of two smaller vicinal couplings for H-1 (eq) requires a 1,3-diaxial arrangement for H-2 and H-10.

It is interesting to note that, while the resonances of hydrogens on C-1 to C-4 of the quinolizidine skelton are very sharp, the resonances of hydrogens on C-6 to C-10 are severely broadened. This indicates that rapid conformational changes are occurring in the unsubstituted ring—most likely by inversion at the nitrogen.

The ^{13}C-nmr spectrum of **2** is easily assigned by comparison with the reported spectrum of the unsubstituted quinolizidine ring system (8) as shown in table 1. As can be seen from the chemical shifts, the two equatorial aryl substituents exert a very symmetrical perturbation on the quinolizidine ring carbons, C-1 to C-4.

TABLE 1. ^{13}C-nmr chemical shift assignments for kayawongine, **2**, in $CDCl_3$.

Atom	(quinolizidine)	(3,4-dimethoxyphenyl)	(4-methoxyphenyl)
C-1	42.3(33.2)[a]	136.7	134.2
C-2	47.8[b](24.4)	110.7[b]	128.5
C-3	48.1[b](25.6)	148.3	113.5
C-4	64.0(56.4)	146.7	157.6
C-5	—	110.6[b]	113.5
C-6	55.8(56.4)	119.3	128.5
C-7	25.7(25.6)		
C-8	24.3(24.4)		
C-9	32.8(33.2)		
C-10	62.4(62.9)		
OCH_3	—	55.4(2C)	54.8

[a]Values in parentheses are literature assignments for the unsubstituted quinolizidine ring system (8).

[b]Values within a column may be interchanged.

The uv spectrum of **2** indicates the presence of two isolated aromatic chromophores. The cd spectrum of **2** does not indicate, however, any exciton split interaction between the chromophores (9), inasmuch as all cd maxima (negative) are centered on the uv maxima. Thus, no evidence on the absolute configuration of **2** is available from the cd spectrum without reference to suitable model compounds. Lacking these models, we choose to draw **2** in this paper in the same 10*R* configuration that has been assigned to cryptopleurine by cd spectral studies (4).

To justify this choice, we cite that the biosynthesis of the phenanthrene nucleus in **1** from a phenolic stilbene such as desmethyljulandine (**6a**),[3] by a dienon-phenol oxidative coupling as suggested by Barton (10) was demonstrated *in vitro* with **6c** by Paton, *et al.* (2) in their synthesis[4] of **1**. In *C. rheifolia*, a phenolic stilbene precursor could lead to both **1** and **2**, giving both molecules identical stereochemistry at C-10.

[3]Compound **6b**, a minor alkaloid accompanying **1** in *Boehmeria platyphylla* (5) and *B. cylindrica* (6), was belatedly named julandine (11).

[4]The paper reporting the synthesis of **1** also describes the preparation of two racemic diastereomers of **2**. However, the stereochemical identities of the two diastereomeric pairs were not assigned (2).

Vomifoliol (**3**) has been isolated from numerous plant families (12-16) and has been synthesized (17,18). Our sample of **3** has physical and spectral properties in full agreement with the literature values.[5]

The allenic ketone (**4**) was first obtained from the oxidation of the carotenoid, fucoxanthin (19). Subsequently, **4** was isolated as a natural product from the defensive spittle of a species of grasshopper, *Romalea microptera* (20) and was named romalea allene. Total syntheses of **4** have been achieved (19, 21). Our isolation of **4** appears to represent the first identification of this compound in the plant kingdom. By comparing the cd spectrum of **4** with the literature values,[6] it is apparent that **4** from *C. rheifolia* has the same absolute configuration as found in **4** isolated from the grasshopper.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Most experimental details have been described (22). Solvent A is a mixture of ethylene dichloride and ethyl acetate of a stated v/v composition. E. Saifah (1978) and Y. Saifah (1979) collected the plants.

PLANT MATERIAL.—*Cissus rheifolia* Planch. (Vitaceae) was collected at Paktongchai in the Province of Nakorn Ratchasima in northeastern Thailand in the late fruiting stage on 19 July 1978, and in the flowering and early fruiting stage on 29 May 1979. The plant was identified by Drs. P. F. Stevens and N. G. Miller at the Arnold Arboretum Herbarium, Cambridge, MA, where voucher specimens have been placed. Chemcial isolations were carried out separately on material from each collection, and compounds **1-5** were isolated both times. We report here our second isolation procedure.

EXTRACTION AND CHROMATOGRAPHY.—Leaves of *C. rheifolia* were dried in an oven (40-50°) and were ground to afford 4.3 kg of powder. This material was extracted by percolation with methanol until the extracts gave a negative Dragendorff's test for alkaloids. The methanolic extract was concentrated to 1.8 liter and was exhaustively extracted with pentane in a liquid-liquid extractor. The 80 g of pentane solubles contained no alkaloids (Dragendorff), gave a positive Liebermann-Burchard test for steroids, but was not further investigated. Filtration of the methanolic solution removed 13.2 g of a yellow solid, rich in flavonoids.

The dark methanolic filtrate was concentrated to 1 liter and was diluted with an equal volume of water. Exhaustive extraction with chloroform gave 54.3 g of an oil. This oil was dissolved in solvent A (9:1), was slurried with 200 g of alumina, and was filtered. The alumina was washed with several portions of ethyl acetate until the washes were colorless. Concentration of the combined washes yielded 11.1 g of a gum. A similar washing of the alumina with methanol gave 18.9 g of a gum, which was Dragendorff-negative and was not further investigated.

Chromatography of the 11.1-g sample on alumina (200 g) in a flat-bottom column (90 mm id) employing solvent A (9:1) gave 2.3 g of crude alkaloid in the first 400 ml of eluate. After elution with an additional 3 liters of the same solvent, the crude terpenoids (0.9 g) were eluted with 0.9 liters of solvent A (8:2) and then with 0.6 liters of solvent A (1:1).

From the 2.3 g of crude alkaloids, 1.46 g of a dark green, gummy solid was obtained on stirring with cyclohexane. This solid was dissolved in ethyl acetate, and the alkaloids were extracted into 5% citric acid solution. Basification of the aqueous extract with concentrated ammonium hydroxide and extraction with ethyl acetate gave 1.05 g of a light-colored gum.

CRYPTOPLEURINE (**1**).—The 1.05 g of gum was dissolved in cyclohexane, and, when left to stand, crystalline **1** (36 mg), mp 182 dec, separated. The ^{1}H-nmr and ms data were obtained. Recrystallization from acetone (6) gave just 2 mg of **1**, mp 193-4 [Lit. (6) mp 195-7]. After evaporation of the acetone, the residue was dissolved in ethanol and treated with picric acid. Crystalline **1**-picrate (4 mg), mp 214-5 [Lit. (23) mp 221-222] was obtained.

KAYAWONGINE (**2**).—The cyclohexane solution from which **1** had crystallized gave, on concentration, crystalline **2** (436 mg, 0.01%) in three crops. Recrystallization from cyclohexane gave **2**, mp 124-124.5, which exhibited the following properties: $[\alpha]^{25}D$ −116 (c=0.85, $CHCl_3$); uv max (ε) (EtOH) 281 sh (4100), 276 (4300), 226)10,200); cd max (EtOH) $(\theta)_{283sh}$ -3280, $(\theta)_{277}$ -4100, $(\theta)_{227}$ -34,800; ^{1}H-nmr (270 MHz, $CDCl_3$) δ 6.99 (d, *J* 9, 2H), 6.69 (d, *J* 9, 2H), 6.63 (d, *J* 9, 1H), 6.60 (m, 2H), 3.77 (s, 3H), 3.75 (s, 3H), 3.71 (s, 3H), 3.10 (td, *J* 11, 4, H-3), 2.96 (dd, *J* 11, 4, H-4 eq), 2.88 (d, broad, *J* 11, H-6 eq), 2.76) td, *J* 11, 4, H-2), 2.28 (t, *J* 11, H-4 ax), 2.08 (m, 1H, H-6 ax), 1.99 (m, 1H, H-10), 1.88

[5]We gratefully acknowledge Professor Koji Nakanishi for providing a copy of the thesis of G. Weiss (18) containing spectral data on synthetic **3**.

[6]We sincerely thank Professor Kenji Mori for copies of the ir and cd spectra of synthetic **4** (21).

(dt *J* 11, 4, H-1 eq), and the following multiplets 1.77 (1H), 1.68 (4H), 1.34 (2H) arising from H-1 ax (at 1.68) and H-7, 8 and 9; ^{13}C-nmr see table 1; ms 381.228 (50%), 380 (24%), 366 (4%), 273.174 (7%), 270.125 (13%), 260.166 (5%), 255.101 (9%), 243.162 (7%), 217.145 (6%), 216.137 (13%), 164.085 (13%), 134.074 (100%), 111.104 (4%), 110.098 (6%), 98.096 ($C_6H_{12}N$, 18%), 96.081 (10%).

Anal. calcd for $C_{24}H_{31}NO_3$ C, 75.56; H, 8.19; N, 3.67. Found: C, 75.88; H, 8.06; N, 3.64.

Attempts to prepare a picrate derivative of **2** did not lead to a crystalline product from ethanol. From a solution of equimolar amounts of **2** and L_g-tartaric acid, however, a derivative was obtained after numerous chilling and thawing cycles. Recrystalization from methanol-ethyl acetate gave **2**-tartrate, mp 172-173.5.

Anal. calcd for $C_{28}H_{37}NO_9$: C, 63.26; H, 7.03; N, 2.64. Found: C, 63.14; H, 6.98; N, 2.65.

VOMIFOLIOL (**3**) AND ROMALEA ALLENE (**4**).—The 0.9 g chromatography fraction containing the terpenoids was subjected to column chromatography and preparative tlc on silica gel. The less-polar, major compound was identified by its ir spectrum,[5] its ^{1}H-nmr spectrum (12), and its ms as **3**. By sublimation (80°/0.01 mm) and recrystallization from acetone-ether, 16 mg of **3**, mp 115-6 [Lit. (12) mp 115] was isolated.

The more polar fractions from the preparative tlc when assayed by ir (film) showed an allenic absorption at 1930 cm^{-1}. By sublimation and recrystalization as above, 8 mg of **4**, mp 129 [Lit. (20) mp 128-128.5] was isolated. Comparison with authentic ir and cd spectra (supplied by Professor Kenji Mori) and with published nmr and ms data (19) confirmed the identification of **4**.

VITEXIN (Apigenin 8-C-glucopyranoside) (**5**).—A portion of the 13.2 g of flavonoid-rich solid was recrystallized from 2-methoxyethanol to yield a yellow solid, mp 246-8. A suspension of this solid (330 mg) was refluxed in 2-propanol-4*N*-HCl (5:2) for 2 h. The solid recovered on filtration (251 mg) melted at 258-9 [Lit. (24) 262-3] and had ir, ^{1}H-nmr, ^{13}C-nmr, and ms data fully consistent with its identity as **5**.

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