BIOLOGICAL AND PHYTOCHEMICAL INVESTIGATION OF *CLAUSENA EXCAVATA*[1]

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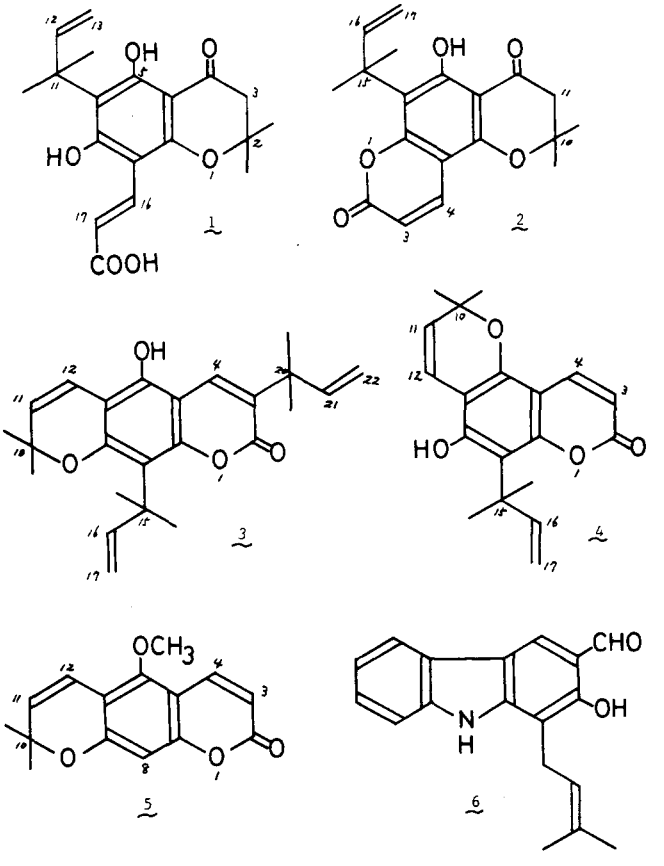
ABSTRACT.—A new compound, clausenidinaric acid (**1**), along with known compounds clausenidin (**2**), clausarin (**3**), nordentatin (**4**), xanthoxyletin (**5**), and heptaphylline (**6**), was isolated from the root bark of *Clausena excavata* (Rutaceae), and its structure was elucidated. In addition, antibacterial activities of these compounds were examined (table 1).

Clausena excavata Burm. f. (*C. lunulata* Hayata) (Rutaceae) is a wild shrub which has been claimed to be a useful folk medicine in the treatment of snake-bite and as a detoxification agent (1). P. K. Bose et al. (2) have reported the isolation of the coumarins clausenidin (**2**) and clausenin from the root and stem bark of *C. excavata*. The isolation of bioactive compounds from this plant was attempted with interest because the crude ethanolic extract of this plant showed antibacterial



effects. We now report the isolation of an antibacterial compound, nordentatin (**4**), along with other known compounds, clausenidin (**2**), clausarin (**3**), xanthoxyletin (**5**), and heptaphylline (**6**), from the root bark of *C. excavata*. The structure elucidation of new component, namely clausenidinaric acid (**1**) is also reported.

[1]Part XIII in the series of "Constituents of Formosan Folk Medicine". For Part XII, see T.S. Wu, C.-S. Kuoh, and H. Furukawa, *Phytochemistry*, **21**, 1771 (1982).

RESULTS AND DISCUSSION

Extraction of the root bark of *C. excavata* with ethanol was followed by liquid-liquid partition of the ethanolic extract between chloroform and water. Crystals of clausenidinaric acid (**1**) were separated from the chloroform extract. The mother liquor of chloroform extract was concentrated and then extracted with hot hexane. The hexane soluble fraction showed strong antibacterial effects. Separation of the hexane fraction by chromatography on silica gel yielded four coumarins, an alkaloid, and sterol mixtures.

Clausenidinaric acid (**1**), colorless needles from acetone, $C_{19}H_{22}O_6$, mp 220–222° showed a greenish black color reaction with $FeCl_3$. Its uv spectrum exhibited an absorption band at 292 nm, and a bathochromic shift with NaOH was observed. The ir spectrum of **1** showed bands at 3340 and 1685 cm^{-1} due to hydroxyl and carbonyl moieties, respectively. The ^{1}H-nmr spectrum revealed a sharp two-proton singlet at δ 2.83 and a six-proton singlet at δ 1.50 due to a methylene adjacent to a carbonyl group and a *gem*-dimethyl group, respectively. The above features indicate clausenidinaric acid contained a 2,2-dimethylchroman-4-one system. Furthermore, a lowfield one proton singlet at δ 13.61 (disappeared on D_2O) was attributable to a strongly hydrogen-bonded hydroxyl proton located at *peri*-position for a carbonyl group. A singlet signal at δ 1.41 corresponding to two equivalent methyl groups and an isolated ABX type signal at δ 6.24, 4.97 and 4.90 ($J_{AX}=16.0$, $J_{BX}=10.0$, and $J_{AB}=2$Hz) were assigned to a 1,1-dimethyl-allyl moiety. AB-type double doublets centered at δ 7.73 and 6.64 (each $J=16$Hz) were assigned to a *trans* disubstituted carbon-carbon double bond. These spectral data were consistent with structure **1** for clausenidinaric acid. Further proof for this structure was obtained by hydrolysis of clausenidin (**2**) with methanolic KOH, which afforded colorless crystals, mp 220–222°, found to be identical with **1** by comparisons of ^{1}H-nmr, ir, and mixed mp.

The above results led us to propose structure **1** for clausenidinaric acid.

Other compounds isolated from the same plant were characterized as clausenidin (**2**) (4,7), clausarin (**3**) (5), nordentatin (**4**) (6), xanthoxyletin (**5**) (8), and heptaphylline (**6**) (3) by comparisons with the authentic samples (mixed mp, ir, ^{1}H-nmr, and ms).

Antibacterial activities of the compounds isolated in this study are shown in table 1. Nordentatin (**4**) showed completely inhibitory effects against of *Bordetella brochiseptica* 4614, *Bacillus subtilis* 6633, *Pneumococcus*, *Staphylococcus*

TABLE 1. Antimicrobial activities of the constituents of *C. excavata*.

Compounds / Organisms (conc. ppm)	Nordentatin (4)					(1)	(2)	(3)	(5)	Classification
	200	100	50	10	5	500	500	500	500	
*Staphylococcus aureus**	−	−	−	−	−	−	−	−	−	Gram-(+)
Bordetella brochiseptica 4614	+	+	+	+	−	−	−	−	−	Gram-(−)
Salmonella gallinarum X-142	−	−	−	−	−	−	−	−	−	Gram-(−)
Escherichia coli 10536	−	−	−	−	−	−	−	−	−	Gram-(+)
Bacillus subtilis 6633	+	+	+	+	−	−	−	−	−	Gram-(+)
*Pneumococcus***	+	+	+	+	±	−	−	−	−	Gram-(+)
Staphylococcus aureus 6538-P	+	+	+	+	±	−	−	−	−	Gram-(+)
Sarcina lutea 9341	+	±	−	−	−	−	−	−	−	Gram-(+)
Pseudomonas aeruginosa NCTC 10490	+	+	+	+	±	−	−	−	−	Gram-(−)
Saccharomyces calshergensis 9080	−	−	−	−	−	−	−	−	−	Fungi
Aspergillus niger	−	−	−	−	−	−	−	−	−	Fungi

*A clinically isolated, penicillin resistant strain.
**A clinically isolated, pathogenic strain.
+Completely growth inhibition, measured after 48 hrs.
±Not complete effective, faint growth occurred after 24 to 48 hrs.
−Ineffective, normal growth occurred after 24 hrs.

aureus 6538–P, and *Pseudomonas aeruginosa* NCTC 10490 at more than 10 ppm concentration.

EXPERIMENTAL[2]

PLANT MATERIAL.—*Clausena excavata* Burm. f. was collected at the Kehg-Ting Tropical Botanical Garden, Ping-Tung, Taiwan, and verified by Prof. C.-S. Kuoh. The specimen is deposited in the Herbarium of Chia-Nan Junior College of Pharmacy, Tainan, Taiwan, Republic of China.

EXTRACTION AND SEPARATION.—The ethanolic extract of the root bark of *C. excavata* (0.9 kg) was treated with chloroform and water. The chloroform layer was concentrated to 100 ml. Colorless crystals, clausenidinaric acid (**1**) (250 mg), were separated and filtered. The mother liquor was evaporated to dryness and 2 liters of hot hexane were added to the residue. The hexane soluble fraction was evaporated and subjected to chromatography on a silica gel column eluted successively with hexane, benzene, benzene-acetone (9:1), benzene-acetone (8:2), and acetone; **2** (3.4 g), **5** (5.1 g), **6** (0.02 g), **4** (0.2 g), **3** (0.25 g) and sterols (0.06 g) were obtained.

CLAUSENIDINARIC ACID (**1**).—Mp 220–222° [References 6 and 9 reported mp 275–277°], colorless crystals from acetone. The $FeCl_3$ test showed a greenish black reaction. λ max nm: 292; λ max (+NaOH) nm: 304, and 334; ν max cm^{-1}: 3340, and 1685. Anal. Calc. for $C_{19}H_{22}O_6$ C, 65.88; H, 6.40. Found C, 65.84; H, 6.52. ^{1}H-nmr (DMSO-d_6) δ: 13.61 (1H, s, C_5-OH), 7.73 (1H, d, J=16 Hz, H-16), 6.64 (1H, d, J=16 Hz, H-17), 6.24 (1H, q, J=10 & 18 Hz, H-12), 4.90 (1H, q, J=2 & 10 Hz, H-13), 4.97 (1H, q, J=2 & 18 Hz, H-13), 2.83 (2H, s, H-3), 1.50 (6H, s, C_8-$(CH_3)_2$), and 1.41 (6H, s, C_{11}-$(CH_3)_2$). The mass spectrum showed fragment peaks at m/z 346 (M^+, 6%), 302(50%), 287(100%), 231(91%), and 135(34%).

CLAUSENIDIN (**2**) (4,7).—Mp 137–138°, yellow prisms from acetone. The $FeCl_3$ test showed a deep violet reaction. Anal. Calc. for $C_{19}H_{20}O_5$ C, 69.50; H, 6.10. Found C, 69.60; H, 6.20.

ALKALINE HYDROLYSIS OF CLAUSENIDIN (**2**).—A solution of clausenidin (**2**) (100 mg) in MeOH (3 ml) and 1% KOH (20 ml) was refluxed for 30 min. When the product was cooled and acidified with dil. HCl, a precipitate was obtained. Recrystalization of the precipitate from acetone afforded colorless crystals, mp 220–222°, which were identified with clausenidinaric acid (**1**) by comparisons with ^{1}H-nmr, ir and mass spectra and mixed mp.

CLAUSARIN (**3**) (5).—Mp 208–210°, colorless rods from acetone. The $FeCl_3$ test showed a deep blue color reaction. Anal. Calc. for $C_{24}H_{28}O_4$ C, 75.76; H, 7.42. Found C, 75.74; H, 7.41.

NORDENTATIN (**4**) (6).—Mp 178–180°, pale yellow prisms from acetone. The $FeCl_3$ test showed a deep blue color reaction. Anal. Calc. for $C_{19}H_{20}O_4$ C, 73.06; H, 6.45. Found C, 72.89; H, 6.45.

XANTHOXYLETIN (**5**) (8).—Mp 132–133°, colorless elongated prisms from acetone. Anal. Calc. for $C_{15}H_{14}O_4$ C, 69.75; H, 5.46. Found C, 69.67; H, 5.44.

HEPTAPHYLLINE (**6**) (3).—Mp 171–172°, yellow needles from ether.

ANTIBACTERIAL SCREENING.—The antibacterial screening was carried out by the same procedures reported in a previous paper (9).

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[2]Mps are uncorrected. ^{1}H-nmr were taken at 100 MHz with a JEOL PS–100 spectrometer in acetone-d_6, except as noted. Chemical shifts are shown in ppm (δ) with TMS as internal standard. Mass spectra were measured with a Hitachi M–52 spectrometer with a direct inlet system. Uv spectra were measured with a JASCO UVIDEC–1 spectrometer in methanol and ir spectra were recorded on a JASCO IRA–1 spectrometer in KBr, except as noted.