THE OCCURRENCE OF TRITERPENOID, PHENOLIC, AND OTHER COMPOUNDS IN THE LEAVES OF SIX ENDEMIC *CASTANOPSIS* SPECIES OF HONG KONG

By H. R. Arthur* and Phyllis D. S. Ko*

[Manuscript received November 1, 1968]

Summary

From the leaves of six *Castanopsis* species of Hong Kong 35 isolations of compounds have been made. These include 12 different triterpenoid compounds (five related to friedelane including the recently isolated canophyllol, two to hopane, glutinol, lupeol, and taraxasterol), ellagic acid, digallic acid, and β-sitosterol.

Introduction

The Fagaceae of Hong Kong are confined to three genera: *Castanopsis*, *Lithocarpus*, and *Quercus*. Four local species of *Quercus* examined before[1,2] yielded a higher alcohol and ketone, β-sitosterol, and triterpenoid compounds related to friedelane and to hopane. *Castanopsis* species from Hong Kong or elsewhere have never previously been examined. Of the ten listed[3] local species, four are rare. Leaves from each of the other six species, all of which were collected from the southern slopes of Hong Kong Island, were extracted successively with light petroleum, acetone, then methanol. From the light petroleum extracts a compound resembling a higher alcohol obtained earlier from *Quercus*[2], all the triterpenoid compounds, and β-sitosterol were obtained; from the acetone extracts, ellagic acid; and from a methanol extract, digallic acid was isolated. The distribution of all compounds found in these species is shown in Table 1. Regarding the triterpenoid compounds, canophyllol (28-hydroxyfriedelan-3-one) recently reported[4] for the first time, from *Calophyllum inophyllum*, was obtained in 0·0006% yield from *Castanopsis concinna*. As well as other triterpenoids actually identified in this plant, a number, which occurred in very low yield in unresolved mixtures, was also present. Although 3β,22-dihydroxyhopane has been obtained before[5,6] by preparation from hydroxyhopanone, its first natural occurrence

* Department of Chemistry, University of Hong Kong, Hong Kong.

[1] Arthur, H. R., Hui, W. H., Lam, C. N., and Szeto, S. K., *Aust. J. Chem.*, 1964, **17**, 697.

[2] Arthur, H. R., Cheng, K. F., Lau, M. P., and Lie, K. J., *Phytochemistry*, 1965, **4**, 969.

[3] Tang, H. C., and Leung, W. T., "Check List of Hong Kong Plants." p. 17. (Urban Council and Urban Services Department: Hong Kong 1967.)

[4] Govindachari, T. R., Viswanathan, N., Pai, B. R., Rao, U. R., and Srinivasan, M., *Tetrahedron*, 1967, **23**, 1901.

[5] Dunstan, W. J., Fazakerly, H., Halsall, T. G., and Jones, E. R. H., *Croat. chem. Acta*, 1957, **29**, 173.

[6] Cerny, J., Vystrcil, A., and Huneck, S., *Chem. Ber.*, 1963, **96**, 3021.

(in *C. eyrei*) was met with in this survey and has been reported.[7] In *C. hickelii* was found in 0·0001% yield, a triterpene acetate, m.p. 310°, ν_{max} 1740, 1262 cm^{-1} (OAc), which was neither α- nor β-friedelanyl acetate nor taraxeryl acetate.

Ten Chinese *Quercus* species are reported by B. E. Read[8] to have medicinal use. Local *Quercus* species are regarded as poisonous; *Castanopsis* and *Lithocarpus* species are not so regarded, and are not used medicinally in Hong Kong.

TABLE 1

COMPOUNDS FROM CASTANOPSIS SPECIES

Compound Isolated	*Castanopsis* Species
Friedelin	all *C.* species
Friedelan-3β-ol	all *C.* species
Friedelan-3α-ol	*C. cuspidata* (Thb.) Schby.
Friedelan-3α-yl acetate	*C. cuspidata*
28-Hydroxyfriedelan-3-one (canophyllol)	*C. concinna* A. DC.
Glutinol	*C. fabri* Hance
22-Hydroxyhopan-3-one	*C. eyrei* (Champ.) Tutch., *C. hickelii* A. Camus
3β,22-Dihydroxyhopane	*C. eyrei*
Lupeol	*C. cuspidata*, *C. fissa* Rehd. and Wils
Taraxasterol	*C. fabri*, *C. hickelii*
β-Sitosterol	all *C. species*
Ellagic acid	*C. concinna*, *C. eyrei*, *C. fabri*
Digallic acid	*C. fissa*

EXPERIMENTAL

Microanalyses were made by the Microanalytical Laboratory, University of Singapore. Specific rotations were measured in chloroform solution. Infrared spectra (taken in Nujol on a Perkin–Elmer 337 spectrophotometer) of all compounds isolated as well as of their derivatives were identical with those of authentic samples. Melting points were taken on a Kofler block. Except for ellagic acid and its derivatives, melting point determinations of all compounds were made in admixture with authentic samples and no depressions were observed. Whatman No. 1 paper was used for paper chromatography and the ascending technique was employed. Ultraviolet spectra were taken on a Unicam SP800 ultraviolet spectrophotometer. Light petroleum had b.p. 60–80°. Except where otherwise stated the alumina used was B.D.H. preparative grade. Gradient elution was employed throughout for column chromatography with solvents in the order: light petroleum; benzene; chloroform. Mixtures of solvents stated for elution were average values e.g. light petroleum–benzene 4 : 1 could mean, typically, 83–77% light petroleum in benzene. All triterpenoid compounds and β-sitosterol were crystallized from light petroleum–benzene except for friedelin, lupeol, lupenone (from light petroleum), glutinyl and lupenyl acetates (from ethanol).

[7] Arthur, H. R., and Ko, Phyllis D. S., *Aust. J. Chem.*, 1968, **21**, 2583.

[8] Read, B. E., "Chinese Medicinal Plants from the Pen Ts'ao Kang Mu." Peking Natural History Society Bulletin, Peking, 1936, p. 196.

Extraction (for all Castanopsis *species)*

Air-dried leaves were milled, then extracted twice with light petroleum at room temperature. The combined extracts were evaporated to dryness; a green residue A was obtained. The leaves were then extracted twice with acetone at room temperature. The combined acetone extracts were concentrated to 2 l. or to dryness. In some cases a pale yellow crystalline solid B separated. The leaves were finally extracted twice with methanol at room temperature. The combined methanol extracts were concentrated to 2 l. or to dryness and gave solid C.

Isolation of Products

(*a*) *From Petroleum Extracts*

Castanopsis concinna.—The residue A (260 g) from 13 kg of leaves was dissolved in light petroleum and applied to a column of alumina (3·0 kg). Gradient elution with: light petroleum–benzene 4 : 1 gave friedelin (1·7 g), m.p. 260°, $[\alpha]_D$ −23·6° (*c*, 0·5); light petroleum–benzene 7 : 3, friedelan-3β-ol (1·0 g), m.p. 290°, $[\alpha]_D$ +26·0° (*c*, 0·66); light petroleum–benzene 1 : 1, a triterpenoid mixture; light petroleum–benzene 1 : 4, β-sitosterol (0·5 g), m.p. 139–140°, $[\alpha]_D$ −35·8° (*c*, 0·62); benzene–chloroform 9 : 1, a triterpenoid alcohol (0·01 g), m.p. 212–216°, ν_{max} 3360 cm^{-1} (OH); benzene–chloroform 6 : 4, 28-hydroxyfriedelan-3-one (canophyllol) (0·08 g), m.p. 280–285°, $[\alpha]_D$ −8·0° (*c*, 0·75).

C. cuspidata.—Residue A (160 g) from leaves (8·0 kg) was treated as stated for *C. concinna.* Alumina used 3·0 kg. Gradient elution with: light petroleum–benzene 9 : 1 gave friedelan-3α-yl acetate (0·9 g), m.p. 310°, $[\alpha]_D$ −10·0° (*c*, 0·4), ν_{max} 1740, 1250 cm^{-1} (OAc); light petroleum–benzene 4 : 1, friedelin (0·03 g), m.p. 260°, $[\alpha]_D$ −29·0° (*c*, 0·60); light petroleum–benzene 7 : 3, friedelan-3β-ol (0·08 g), m.p. 290°, $[\alpha]_D$ +25·9° (*c*, 0·54); light petroleum–benzene 1 : 1, lupeol (1·5 g), m.p. 203–212°, $[\alpha]_D$ +25·0° (*c*, 0·62), ν_{max} 3450 cm^{-1} (OH), 1650, 884 cm^{-1} (>C=CH$_2$), which was characterized as the acetate, m.p. 218–219°, ν_{max} 1740, 1245 cm^{-1} (OAc), and as lupenone, m.p. 168–170°, ν_{max} 1720 cm^{-1} (CO); light petroleum–benzene 1 : 1, friedelan-3α-ol (0·65 g), m.p. 307°, $[\alpha]_D$ +15·0° (*c*, 0·40); light petroleum–benzene 3 : 7, β-sitosterol (1·1 g), m.p. 138–139°, $[\alpha]_D$ −37·2 (*c*, 0·60), ν_{max} 3310 cm^{-1} (OH).

C. eyrei.—Residue A (100 g) from leaves (7 kg). Alumina used 2·5 kg. Gradient elution with: light petroleum–benzene 7 : 3 gave friedelin (1·65 g), m.p. 262–264°, $[\alpha]_D$ −21·5° (*c*, 0·4); light petroleum–benzene 1 : 1, friedelan-3β-ol (0·9 g), m.p. 289–290°, $[\alpha]_D$ +25·1 (*c*, 0·64); benzene–chloroform 9 : 1, β-sitosterol (0·25 g), m.p. 139°, $[\alpha]_D$ −36·9° (*c*, 0·68); benzene–chloroform 7 : 3, 22-hydroxyhopan-3-one (0·04 g), m.p. 250–254°, $[\alpha]_D$ +62·9° (*c*, 0·82); benzene–chloroform 3 : 7, 3β,22-dihydroxyhopane (0·02 g), m.p. 285–286°, $[\alpha]_D$ +36·7° (*c*, 0·52).

C. fabri.—Residue A (100 g) from leaves (6 kg). Alumina used 2·5 kg. Gradient elution with: light petroleum–benzene 9 : 1 gave friedelin (0·9 g), m.p. 262°, $[\alpha]_D$ −29·1° (*c*, 0·68); light petroleum–benzene 6 : 4, friedelan-3β-ol (2·0 g), m.p. 280–285°, $[\alpha]_D$ +24·0 (*c*, 0·60); light petroleum–benzene 1 : 1, glutinol (0·5 g), m.p. 210–212°, $[\alpha]_D$ +54·7° (*c*, 0·73); ν_{max} 3480 cm^{-1} (OH), which was characterized as the acetate, m.p. 190–192°, ν_{max} 1740, 1250 cm^{-1} (OAc); light petroleum–benzene 2 : 3, taraxasterol (0·02 g), m.p. 220°, $[\alpha]_D$ +91·5° (*c*, 0·53); light petroleum–benzene 1 : 4, β-sitosterol (0·4 g), m.p. 139°, $[\alpha]_D$ −34·0° (*c*, 0·81), ν_{max} 3310 cm^{-1} (OH).

C. fissa.—Residue A (103 g) from leaves (6·4 kg). Alumina (Spence grade 0 deactivated with 10% HOAc, 5 ml/100 g) used 3·2 kg. Gradient elution with: light petroleum–benzene 1 : 1 gave friedelin (0·1 g), m.p. 260°, $[\alpha]_D$ −26·2° (*c*, 0·56); then friedelan-3β-ol (2·0 g), m.p. 282°, $[\alpha]_D$ +26·1° (*c*, 0·50); then a mixture which yielded on acetylation lupenyl acetate, m.p. 219°; then β-sitosterol (0·4 g), m.p. 138°, $[\alpha]_D$ −34·4° (*c*, 0·62).

C. hickelii.—Residue A (130 g) from leaves (7 kg). Alumina used 2·5 kg. Gradient elution with: light petroleum–benzene 4 : 1 gave a triterpenoid acetate, m.p. 310°, ν_{max} 1740, 1262 cm^{-1} (OAc); light petroleum–benzene 7 : 3, friedelin (5·0 g), m.p. 262°, $[\alpha]_D$ −26·1° (*c*, 0·61); light petroleum–benzene 1 : 1, friedelan-3β-ol (3·6 g), m.p. 290°, $[\alpha]_D$ +23·0° (*c*, 0·53); light petroleum–benzene 1 : 4, taraxasterol (0·4 g), m.p. 220–223°, $[\alpha]_D$ +99·5° (*c*, 1·27), which was characterized as the acetate, m.p. 243–246°, ν_{max} 1740, 1250 cm^{-1} (OAc); benzene–chloroform

4 : 1, β-sitosterol (1·3 g), m.p. 139–140°, $[\alpha]_D$ −36·8° (*c*, 0·66); benzene–chloroform 6 : 4, 22-hydroxyhopan-3-one (0·4 g), m.p. 245°, $[\alpha]_D$ +55·0° (*c*, 0·66).

(*b*) *From Acetone Extracts*

Castanopsis concinna.—Product B (3·2 g), a mixture, contained ellagic acid which was characterized as ellagic acid tetraacetate, m.p. 340° after recrystallization from acetic anhydride.

C. cuspidata.—Product B was not identified.

C. eyrei.—Product B (3·0 g), a mixture, contained ellagic acid which was isolated as the tetraacetate, m.p. 340° after crystallization from acetic anhydride.

C. fabri.—Product B (5·0 g) was ellagic acid, m.p. > 360°, ν_{max} 3560 cm^{-1} (OH), 1720 cm^{-1} (CO of lactone), 1520 (aromatic rings), after crystallization from pyridine–ethanol. It was characterized as the tetraacetate as stated above.

C. fissa.—Product B was a tar.

C. hickellii.—Product B was not identified.

(*c*) *From Methanol Extracts*

C. fissa.—Product C (95 g), m.p. 285–287° (vac., dec.) after crystallization from aqueous ethanol, gave digallic acid (*m*-digallic acid), m.p. 285–287°; λ_{max} (log ε) 226 (4·18), 275 (4·15); R_F 0·33 (Bu^nOH–AcOH–H_2O, 4 : 1 : 5). Digallic acid was characterized as the pentaacetate, m.p. 214–216° after crystallization from methanol, and by hydrolysis (9N sulphuric acid solution on a steam-bath for 3 hr) into gallic acid, m.p. 253–255° (vac., dec.) after crystallization from water, which had R_F 0·59 (Bu^nOH–AcOH–H_2O, 4 : 1 : 5); 0·57 (H_2O saturated with phenol) (Found: C, 49·5; H, 3·95. Calc. for $C_7H_6O_5$: C, 49·4; H, 3·6).

C. coninna, *C. cuspidata*, *C. fabri*, *C. hickelii*.—In each case solid C could not be identified.

C. eyrei.—Product C was a tar.

Acknowledgments

The authors thank Dr T. G. Halsall, Dyson Perrins Laboratory, Oxford, and Dr T. R. Govindachari, Director, Ciba Research Centre, Bombay, for triterpenoid samples; Mr H. C. Tang, Government Herbarium, Hong Kong, for identifying the plants; the Research Grants Committee of the University of Hong Kong for a grant-in-aid; and the Government of Hong Kong for a Postgraduate Studentship for one of us (P.D.S.K.).