Phytochemistry, 1976, Vol. 15, pp. 1313–1315. Pergamon Press. Printed in England.

TERPENOIDS AND STEROIDS FROM *CASTANOPSIS LAMONTII*: A NEW TRITERPENE KETOL

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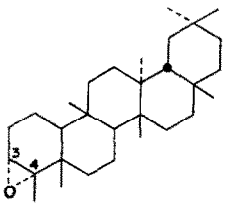
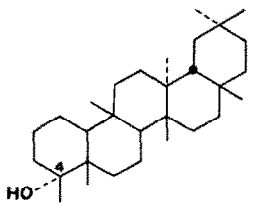
(*Received* 12 *February* 1976)

Key Word Index—*Castanopsis lamontii*; Fagaceae; diterpenoid, 6,20-epoxylathyrol-5,10-diacetate-3-phenylacetate; triterpenoids, 15α-hydroxyolean-12-en-3-one, methyl oleanolate, friedelane derivatives, taraxerol, betulin; steroids, 6β-hydroxystigmast-4-en-3-one, sitosterol.

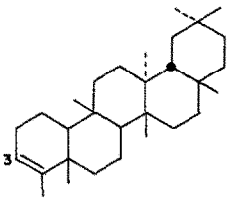
Previous work on six local *Castanopsis* species [1–3] led to the isolation of a number of triterpenoids and two steroids. The present investigation is a study of the seventh species, *C. lamontii*. Column chromatography on alumina of the petrol extract of the leaves gave the triterpenoids friedelin, friedelan-3β-ol, taraxerol, betulin, and sitosterol. The extract from the stems gave the same compounds except for betulin, and also the triterpenoids, 3α,4α-epoxyfriedelane (**1**), methyl oleanolate, canophyllol, a new compound 15α-hydroxyolean-12-en-3-one (**4**), the steroid, 6β-hydroxystigmast-4-en-3-one, and the diterpenoid derivative, 6,20-epoxylathyrol-5,10-diacetate-3-phenylacetate. The last compound has only been isolated twice previously, on both occasions from Euphorbiaceae species [4]. Both the leaves and stems, after extraction with petrol, were further extracted with 95% EtOH, and tested for acidic triterpenoids. Negative results were obtained.



(**1**)



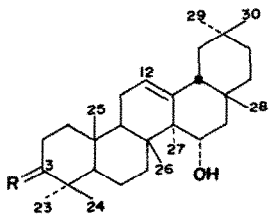
(**2**)



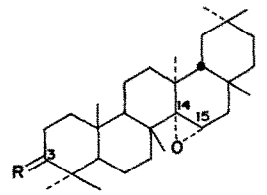
(**3**)

3α,4α-Epoxyfriedelane (**1**), $C_{30}H_{50}O$, showed evidence of a 1,2-epoxy ring in its IR and NMR spectra. On treating with HCl in $CHCl_3$–MeOH solution for 20 hr **1** yielded a mixture, separable by PLC to give friedelin as the major product, the minor products being a mixture of alcohols. Complete isomerization to friedelin was achieved by shaking a solution in $CHCl_3$ with alumina for 7 days. On $LiAlH_4$ reduction, **1** gave a saturated tertiary alcohol $C_{30}H_{52}O$ (**2**), which on dehydration with $POCl_3$ in excess of pyridine afforded friedel-3-ene (**3**). Thus the OH group in **2** must be at C-4 and axially (α) orientated, and **2** is therefore friedelan-4α-ol. Compound **1** was finally proved to be 3α,4α-epoxyfriedelane by its identity with a sample of the latter prepared by treatment of friedel-3-ene (**3**) with *m*-chloroperbenzoic acid [5]. 3α,4α-epoxyfriedelane (**1**) has not been isolated previously as a natural product, though its synthesis has been reported [5], and a similar compound, 3α,4α-epoxyfilicane, has been obtained from the fern *Adiantum capillus-veneris* [6]. As **1** could be isomerized to friedelin by alumina, it was considered possible that the friedelin obtained from column chromatography might be an artifact. This was proved not to be the case by similar chromatography of **1**, when no friedelin was eluted.

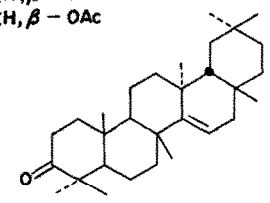
Compound **4**, $C_{30}H_{48}O_2$ (M^+ at *m/e* 440), gave positive results to both the tetranitromethane test and Liebermann–Burchardt reaction. Its IR and NMR spectra indicated a secondary equatorial OH group [ν_{max} 3570 cm^{-1}, δ 4.27 (1H, *q*, $J_{ax/eq}$ 6 Hz, $J_{ax/ax}$ 10 Hz)], a CH_2CO function (ν_{max} 1715 cm^{-1}, δ 2.4 (2H, *m*)], and a trisubstituted double bond [ν_{max} 3050, 1670, 830 cm^{-1}, δ 5.34 (1H, *q*, *J* 3 and 4 Hz)]. The latter together with 8 tertiary methyl signals at δ 0.88–1.18 in its NMR spectrum suggested an olean-12-ene skeleton. The MS of **4** showed characteristic peaks at *m/e*, 234 (100), 219 (37), 216 (13), 205 (28) and 149 (68%), indicating the C=O



(**4**) R = O
(**5**) R = αH, β – OH
(**6**) R = αH, β – OAc



(**7**) R = α – H, β – OAc
(**8**) R = O



(**9**)

function in rings A or B, probably at the usual C-3 position, and the equatorial OH group in ring D [7], either at the C-15α or C-16β position, possibly the former, since the CHOH proton absorbed at fairly low field. The structure for **4** was thus proposed to be 15α-hydroxyolean-12-en-3-one, and this was proved by its reduction

Table 1. NMR Chemical shifts (δ) for the methyl group protons of compounds **4**, **5**, and **6**

	C-23	C-24	C-25	C-26	C-27	C-28	C-29	C-30
(4)	1.09	1.05	1.09	1.09	1.18	0.88	0.88	0.88
(5)	0.99	0.79	0.95	1.05	1.17	0.88	0.88	0.88
(6)	0.88	0.88	0.97	1.02	1.14	0.88	0.88	0.88

to a diol, $C_{30}H_{50}O_2$ (**5**), identical with 3β,15α-dihydroxyolean-12-ene (**5**) prepared from 14α,15α-epoxytaraxeran-3β-yl acetate (**7**) [8], and confirmed by its partial synthesis by acid treatment of 14α,15α-epoxytaraxeran-3-one (**8**) prepared from taraxerone (**9**).

The NMR shifts of the methyl group protons in compounds **4**, **5** and **6** have been assigned as shown in Table 1.

EXPERIMENTAL

IR spectra were recorded for KBr discs; NMR spectra in $CDCl_3$ were determined at 60 MHz using TMS as internal standard; UV spectrum in 95% EtOH, and optical rotations in $CHCl_3$ solns. Petrol had bp 60–80°. Known compounds were identified by TLC, mmp, IR and MS comparisons with authentic samples.

Extraction and isolation of compounds. Milled air-dried leaves and stems of *C. lamontii* were separately extracted 2× at room temp. with petrol for 7 days. Each of the combined extracts was distilled to a small volume and chromatographed on alumina.

Leaves. The extract from leaves (29 kg) was separated on alumina (6 kg). Elution with petrol gave friedelin (3.0 g), mp 260–261°, IR ν_{max} cm^{-1}: 1715, then friedelan-3β-ol (1.0 g), mp 281–284°, IR ν_{max} cm^{-1}: 3630; with petrol–C_6H_6 (1:1), taraxerol (3.0 g), mp 287–289°, IR ν_{max} cm^{-1}: 3500, 3060, 1645, 820, then sitosterol (1.0 g), mp 138–140°; and finally with C_6H_6, betulin (0.01 g), mp 251–253°, IR ν_{max} cm^{-1}: 3400, 3050, 1650, 880.

Stems. The stem (24 kg) extract was chromatographed on alumina (3.5 kg). Elution with petrol gave 3α,4α-epoxyfriedelane (**1**) (0.07 g), mp 231–233° (from $CHCl_3$–MeOH), $[\alpha]_D$ +52.7° (Found: C, 84.6; H, 11.6; M^+ 426. Calc. for $C_{30}H_{50}O$: C, 84.4; H, 11.8%; M^+ 426), IR ν_{max} cm^{-1}: 864, 763 (epoxy), NMR: δ 2.85 (1H, *t*, *J* 2 and 3 Hz, C-3βH), then friedelin (3.1 g), followed by friedelan-3β-ol (0.8 g); with petrol–C_6H_6 (1:1), taraxerol (1.0 g) and sitosterol (2.0 g); with C_6H_6, methyl oleanolate (0.03 g), mp 198–201° (from C_6H_6), MS: *m/e* 470, IR ν_{max} cm^{-1}: 3380, 1730, 1160, 3030, 1650, 840; then canophyllol (0.02 g), mp 279–280° (from C_6H_6), $[\alpha]_D$ −9.0°, IR ν_{max} cm^{-1}: 3550, 1720, followed by 6,20-epoxylathyrol-5,10-diacetate-3-phenylacetate (0.2 g), mp 204–207° (from petrol), MS: *m/e* 552 (M^+) $C_{32}H_{40}O_8$, IR ν_{max} cm^{-1}: 1730, 1260 (RCOO–), 1660, 1620 (>C=C–C=O), 1600, 1580, 730, 700 (mono-substituted C_6H_6 ring); finally with C_6H_6–$CHCl_3$ (1:1), needles of **4** (0.04 g), mp 187–189° (from aq. MeOH), $[\alpha]_D$ +93.0° (Found: C, 81.7; H, 11.1; M^+ 440. $C_{30}H_{48}O_2$ requires C, 81.8, H, 11.0%; M^+ 440), then plates of 6β-hydroxystigmast-4-en-3-one (0.03 g), mp 211–212°, $[\alpha]_D$ +27.5°, MS: *m/e* 428 (M^+) $C_{29}H_{48}O_2$, IR ν_{max} cm^{-1}: 3510 (OH), 1695, 1620 (>C=C–C=O), λ_{max}: 246 nm (ϵ12,900).

Isomerization of **1** (a) A soln of **1** (0.1 g) in $CHCl_3$ (8 ml), MeOH (15 ml) and aq HCl (5M, 5 ml) was kept at room temp for 20 hr. The product was separated by PLC [$CHCl_3$–EtOAc (99:1)] into unchanged **1** (5 mg), friedelin (0.06 g), mp 262–264°. ν_{max} cm^{-1} 1720 (C=O), and a mixture (3 mg), ν_{max} cm^{-1} 3300 (OH). (b) **1** (0.02 g) in $CHCl_3$ (10 ml) was stirred with alumina (5 g) at room temp for 7 days. The soln on concentration gave friedelin (0.017 g).

Reduction of **1**. Compound **1** (0.04 g) in dry THF (25 ml) was refluxed with $LiAlH_4$ (0.03 g) for 16 hr. The product was recrystallized from $CHCl_3$–MeOH to give plates of **2**, (0.035 g), mp 258–260°, $[\alpha]_D$ +67.0°, (Found: C, 84.2; H, 11.9; M^+ 428. $C_{30}H_{52}O$ requires C, 84.0; H, 12.2%; M^+ 428), IR ν_{max} cm^{-1}: 3640, 3490, 1180 (OH).

Dehydration of **2**. Compound **2** (0.03 g) was refluxed with $POCl_3$ (2 ml) in C_5H_5N (25 ml) for 1 hr. The product (0.027 g), mp 261–263° (from petrol), MS: *m/e* 410 (M^+) $C_{30}H_{50}$, IR ν_{max} cm^{-1}: 1670, 840 (>C=CH), was identical with friedel-3-ene (**3**).

Partial synthesis of **1** *from* **3**. **3** (0.1 g) was treated with a soln of freshly purified *m*-chloroperbenzoic acid (0.18 g) in $CHCl_3$ (25 ml) at 0° for 19 hr. The product, 3α,4α-epoxyfriedelane (0.09 g), mp 231–233° (from $CHCl_3$–MeOH), $[\alpha]_D$ +58.0°, MS: *m/e* 426 (M^+) $C_{30}H_{50}O$, was identical with **1**.

Chromatography of **1**. Compound **1** (0.015 g) in petrol was chromatographed on alumina (8 g). Elution with petrol gave unchanged **1** (0.014 g), mp 227–229°.

Reduction of **4**. A soln of **4**, (0.02 g) and $NaBH_4$ (0.025 g) in $(CH_3)_2CHOH$ (25 ml) was stirred at room temp for 2 hr. The product **5** (0.015 g), mp 244–246° (from aq. MeOH), $[\alpha]_D$ +84.0°, MS: *m/e* 442 (M^+) $C_{30}H_{50}O_2$, IR ν_{max} cm^{-1}: 3330 (OH) 3070, 1670, 830 (>C=CH), was identical with 3β,15α-dihydroxyolean-12-ene (**5**) prepared as described below.

3β,15α-dihydroxyolean-12-ene (**5**) *from 14α,15α-epoxytaraxeran-3β-yl acetate* (**7**) [8]. **7** (0.02 g) in MeOH (35 ml) and $CHCl_3$ (10 ml) was treated with aq HCl (5M, 5 ml) at room temp for 18 hr to give plates of 3β-acetoxy-15α-hydroxyolean-12-ene (**10**) (0.015 g), mp 286–288° (from $CHCl_3$–MeOH), $[\alpha]_D$ +76.0°, MS: *m/e* 484 (M^+) $C_{32}H_{52}O_3$. IR ν_{max} cm^{-1}: 3540 (OH), 1720, 1270 (OAc), 1650, 815 (>C=CH), NMR: δ 4.22 (1H, *q*, $J_{ax/eq}$ 6 Hz, $J_{ax/ax}$ 10 Hz, C-15βH), 4.47 (1H, *q*, $J_{ax/eq}$ 7 Hz, $J_{ax/ax}$ 9 Hz, C-3αH), 5.29 (1H, *q*, *J* 3 and 4 Hz, C-12 olefinic H), which was refluxed with $LiAlH_4$ in dry Et_2O (25 ml) for 3 hr to give plates of 3β,15α-dihydroxyolean-12-ene (**5**) (0.012 g), mp 243–246° (from aq MeOH), $[\alpha]_D$ +84.0°, MS: *m/e* 442 (M^+) $C_{30}H_{50}O_2$, IR ν_{max} cm^{-1}: 3330 (OH), 3070, 1670, 830 (>C=CH), NMR: δ 3.22 (1H, *q*, $J_{ax/eq}$ 7 Hz, $J_{ax/ax}$ 9 Hz, C-3αH), 4.27 (1H, *q*, $J_{ax/eq}$ 6 Hz, $J_{ax/ax}$ 10 Hz, C-15βH), 5.32 (1H, *q*, *J* 3 and 4 Hz, C-12 olefinic H).

Partial synthesis of **4**. Taraxerone (**9**) (0.1 g) was treated with freshly purified *m*-chloroperbenzoic acid (0.15 g) and Na_2HPO_4 (5 mg) in $CHCl_3$ (25 ml) at 0° for 18 hr to give prisms of 14α,15α-epoxytaraxeran-3-one (**8**), (0.8 g), mp 197–199° (from $CHCl_3$–MeOH), $[\alpha]_D$ +53.0° (Found: C, 81.7; H, 11.0; M^+ 440. $C_{30}H_{48}O_2$ requires C, 81.8; H, 11.0%; M^+ 440), IR ν_{max} cm^{-1}: 1720 (C=O), 890, 860 (epoxy), NMR: δ 3.08 (1H, *d*, *J* 7 Hz, C-15βH), a soln of which in MeOH (40 ml) and $CHCl_3$ (10 ml) was treated with aq HCl (5M, 20 ml) at room temp for 20 hr. The product, 15α-hydroxyolean-12-en-3-one (0.05 g), mp 187–189° (from aq MeOH), $[\alpha]_D$ +93.0°, MS: *m/e* 440 (M^+) $C_{30}H_{48}O_2$, IR ν_{max} cm^{-1}: 3570, 3050, 1715, 1670, 830, was identical with (**4**).

Acknowledgements—The authors wish to thank the staff of the Government Herbarium, Hong Kong, for identification of plant material, and the Committee on Higher Degrees and Research Grants, University of Hong Kong, for financial assistance.

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Phytochemistry, 1976, Vol. 15, pp. 1315–1317. Pergamon Press. Printed in England.

PHYTOLACCOSIDE B: TRITERPENE GLUCOSIDE FROM *PHYTOLACCA AMERICANA*

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(*Received* 19 *February* 1976)

Key Word Index—*Phytolacca americana*; Phytolaccaceae; poke-weed; triterpenoid glucoside; phytolaccoside B.

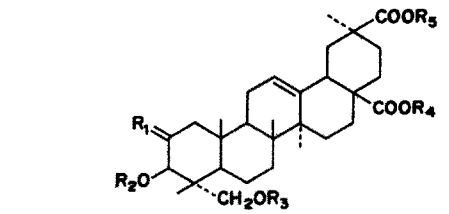
Abstract—The structure of phytolaccoside B, one of the major saponin components of the roots of *Phytolacca americana*, has been elucidated as 3β-D-glucopyranosyljaligonic acid 30-methyl ester.

The roots of *Phytolacca americana* are reputed in Korean medicine to alleviate rheumatism. They were found rich in saponins with strong anti-inflammatory activity [1]. According to earlier work [2,3] hydrolysis of the total saponin mixture yielded four sapogenins, jaligonic acid (**1**) [4] and its 30-methylester (**2**, phytolaccagenin) [5], and esculentic acid (**3**) [6] and its 30-methylester (**4**, phytolaccagenic acid) [7]. This paper describes the structural elucidation of phytolaccoside B, one of the major saponins isolated from the roots of this plant.

Phytolaccoside B (**5**), $C_{37}H_{58}O_{12}$, mp 215–218°, $[\alpha]_D^{25}$ + 75.8°, on acid hydrolysis, gave jaligonic acid 30-methylester (**2**), mp 317–319°, as the genin, which was identified by direct comparison with an authentic sample (mmp, TLC, IR and MS). Glucose was identified in the hydrolysate from the saponin (**5**) by TLC. The yield of the genin in a quantitative hydrolysis experiment showed the presence of one sugar unit in the molecule (genin found 73%; calc. 76.66%).

Methylation of **5** with CH_2N_2 gave methylated product **6**, mp 182–184°, $[\alpha]_D^{25}$ + 71.6°. Saponification of **5** with alkali afforded a new glucoside **7**, mp 234–239°, $[\alpha]_D^{25}$ + 77°, which gave **6** by methylation with CH_2N_2. These results show that the sugar must be present in glucosidic linkage with one of the hydroxyl groups in ring A of the genin (not in ester linkage with the 28-carboxyl group).

In a quantitative periodate oxidation experiment 2 moles of the reagent were consumed. This indicates that the sugar is attached to the C–2 or C–3 position of the genin.



(**1**) R_1 =  ; $R_2 = R_3 = R_4 = R_5 = H$

(**2**) R_1 =  ; $R_2 = R_3 = R_4 = H$; $R_5 = Me$

(**3**) $R_1 = H_2$; $R_2 = R_3 = R_4 = R_5 = H$

(**4**) $R_1 = H_2$; $R_2 = R_3 = R_4 = H$; $R_5 = Me$

(**5**) R_1 =  ; R_2 = Glu; $R_3 = R_4 = H$; $R_5 = Me$

(**6**) R_1 =  ; R_2 = Glu; $R_3 = H$; $R_4 = R_5 = Me$

(**7**) R_1 =  ; R_2 = Glu; $R_3 = R_4 = R_5 = H$

(**8**) R_1 =  ; R_2 = Glu(Ac)$_4$; R_3 = Ac; $R_4 = H$; $R_5 = Me$

(**9**) R_1 =  ; R_2 = Glu(Ac)$_4$; R_3 = Ac; $R_4 = H$; $R_5 = Me$

(**10**) R_1 =  ; R_2 = Glu; $R_3 = R_4 = H$; $R_5 = Me$