Table 1. Composition of the phloroglucinol derivatives in *D. chrysocoma*, *D. filix-mas* and *P. squarrosum*

Taxon and ploidy	Origin	Dried rhizomes (g)	Oleoresin g	(%)	Crude filicin g	(%)	Albaspidin	Filixic acid	Paraaspidin	Desaspidin	Trisdesaspidin	Flavaspidic acid
D. chrysocoma [2x]	India	240	32	(13)	5·3	(2·2)	++	++	−	−	−	+++
D. filix-mas [4x]	Finland	733	74·8	(9·8)	12·7	(1·8)	−	+++	+	+	(+)	+++
P. squarrosum [2x]	India	500	7·2	(1·2)	−	−	−	−	−	−	−	−

Phloroglucinol derivatives in *D. chrysocoma* and *D. filix-mas* exist as mixtures of butyryl (B), propionyl (P), and acetyl (A) homologues (6:3:1) (cf. text). Key:— absent; (+) present in traces (<5%); + present in small amounts (5–10%); + + present in moderate amounts (10–20%); + + + present in large amounts 20%).

vage [3,10] revealed the presence of butyryl (B) (60%), propionyl (P) (30%) and acetyl (A) (10%) homologues in *D. chrysocoma*. Similar percentages were found for *D. filix-mas*.

The presence of filixic acid in *D. chrysocoma* indicates that it is chemotaxonomically related to the taxa of *D. filix-mas* and *D. villarii* complex (cf. Ref. [1]). The high yield of crude filicin, as well as its chemical resemblance to that of *D. filix-mas* confirm its use as a substitute of male fern and justifies its inclusion in Pharmacopoeia of India.

EXPERIMENTAL

Plant Material. Ferns were collected from Simla, Western Himalayas at 1800 m in September 1973. Voucher specimens have been deposited in the Botany Dept. herbarium, University of Helsinki.

Extraction of rhizomes. The powdered rhizomes were macerated 3 × with peroxide free ether and crude filicin obtained with MgO, using Na_2SO_3 as an antioxidant [1–5]. Yields of oleo-resin (Et_2O extracts) and crude filicins are listed in Table 1.

Separation of phloroglucinol derivatives. Crude filicin (5·3 g) was suspended in C_6H_6 and chromatographed on a column containing 130 g Si gel. Fractions 1–4 (10 ml each), eluted with C_6H_6, contained albaspidin and filixic acid. The residue was crystallised from Me_2CO to give 12 mg albaspidin, mp 142–143°, 4 mg albaspidin mp 134–136°, 11 mg filixic acid mp 169–170° and 17 mg crystalline mixture of filixic acid and albaspidin mp 112–116°. Fractions 5–35 eluted with C_6H_6 contained albaspidin, filixic acid and some flavaspidic acid. On cryst. from Me_2CO 2 mg albaspidin mp 133–134° and 17 mg crystalline mixture of albaspidin and filixic acid mp 84–86° were obtained. Fractions 36–95 eluted with C_6H_6–$CHCl_3$ (1:1) contained only flavaspidic acid. On cryst. from Me, 34 mg flavaspidic acid BB mp 150–152° and 5 mg more crystals mp 115–120° were obtained. The rest of this fraction (2·15 g) consisted of a brown oil, which did not crystallize.

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REFERENCES

1. Widén, C.-J., Vida, G., von Euw, J. and Reichstein, T. (1971) *Helv. Chim. Acta* **54**, 2824.
2. Britton, D. M. and Widén, C.-J. (1974) *Can. J. Botany* **52**, 627.
3. Widén, C.-J., Faden, R. B., Lounasmaa, M., Vida, G., von Euw, J. and Reichstein, T. (1973) *Helv. Chim. Acta* **56**, 2125.
4. Widén, C.-J., Lounasmaa, M., Vida, G. and Reichstein, T. (1975) *Helv. Chim. Acta* **58**, 880.
5. Widén, C.-J., Lounasmaa M. and Sarvela, J. (1975) *Planta Med.* **28**, (in press).
6. Mittal, T. C. and Mehra P. N. (1960) *J. Pharm. Pharmacol.* **12**, 317.
7. Mehra, P. N. and Mittal, T. C. (1961) *J. Pharm. Pharmacol.* **13**, 300.
8. Puri, H. S. (1970) *Am. Fern J.* **60**, 137.
9. Handa, K. L., Kapoor, L. D. and Singh, H. (1952) *Indian J. Pharm.* **14**, 109.
10. Widén, C.-J. (1968) *Farm. Aikak.* **77**, 30.

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LINDLEYIN, A NEW PHENOLIC GALLYLGLUCOSIDE FROM *AEONIUM LINDLEYI*

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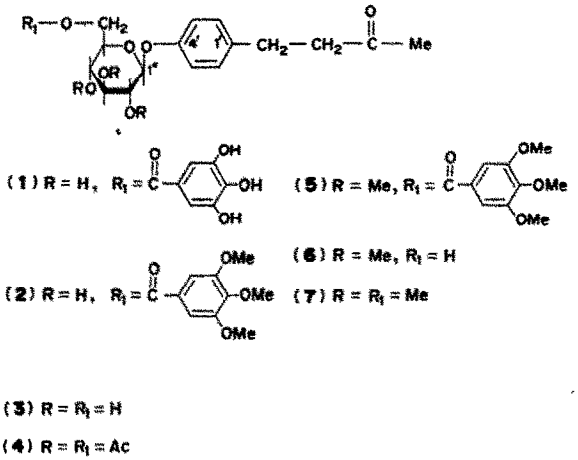
Key Word Index—*Aeonium lindleyi*; Crassulaceae; glucoside; lindleyin; 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-β-D-(6″-*O*-gallyl)glucoside.

In a previous study of *Aeonium lindleyi* W. B., labdane-8α, 15-diol was obtained [1]. The present work reports the isolation of a new phenolic gallylglucoside, named lindleyin, which on the basis of spectral data and

chemical transformations was assigned the structure of 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-β-D-(6″-*O*-gallyl)-glucopyranoside (**1**).

By microanalysis and high resolution MS lindleyin (**1**), mp 210–211°, had the molecular formula $C_{23}H_{26}O_{11}$ [*m/e* 460·1377 ($M^+ - H_2O$)]. Treatment with CH_2N_2 gave (**2**) ($C_{26}H_{32}O_{11}$; M^+ 520) the MS and NMR spectrum of which showed that three phenolic hydroxyls had been methylated [singlets at δ 3·85 (6H) and 3·94 (3H)]. Methanolysis of (**2**) with Na_2CO_3–MeOH yielded tri-*O*-methylgallic acid methyl ester, identified by comparison with an authentic sample, and a glucoside (**3**) ($C_{16}H_{22}O_7$; M^+ 326) that presented IR absorptions of hydroxyl and carbonyl functions (ν_{max} 3480, 1710 cm^{-1}) and phenyl ring (ν_{max} 3050, 1610, 1510 cm^{-1}). In its MS the base peak at *m/e* 164 and the fragments at *m/e* 107, 91 and 43 corresponded to the aglucone moiety and indicated that it must have the structure of 4-hydroxyphenyl-2-butanone. Mild acetylation of (**3**) gave (**4**) ($C_{24}H_{30}O_{11}$) whose NMR spectrum displayed 2 singlets for 4 acetate groups and a 4-proton A_2B_2 system for a *p*-disubstituted benzene ring. By acid hydrolysis of (**3**) glucose and 4-(*p*-hydroxyphenyl)-2-butanone were obtained; the latter was identified by means of a sample synthesized from 3-(*p*-hydroxyphenyl)propionic acid with MeLi [2]. The above results together with the fact that the glucoside (**3**) was also hydrolyzed by β-glucosidase established its structure as 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-β-D-glucopyranoside.

In order to determine the position of the gallyl moiety in lindleyin (**1**), (**2**) was permethylated [3] to (**5**), which lacked IR bands for hydroxyls and presented NMR signals for 6 methoxy groups. Methanolysis of (**5**) gave (**6**) that possessed OH functions (ν_{max} 3600 cm^{-1}), the parent ion in its MS appearing at *m/e* 368. The NMR spectrum ($CDCl_3$, 240 MHz) displayed a three-protons singlet of a methylketone at δ 2·10, 2 singlets corresponding to 3 MeO groups at δ 3·58 (3H) and 3·65 (6H), a doublet of the anomeric proton at C-1″ (δ 4·88, *J* 8 Hz), and a 4-proton A_2B_2 system of the *p*-disubstituted benzene ring (δ 6·89, 6·92, 7·14, 7·17). The protons at C-3 and C-4 were present as 4 peaks at δ 2·70, 2·73, 2·80 and 2·83 which in C_6D_6 were transformed into an A_2X_2 system (δ 2·24, 2·80, *J* 8 Hz). The methoxy singlets overlapped with a 6-proton multiplet of the remaining glucose hydrogens. This multiplet was partially resolved with the aid of $Eu(fod)_3$ (20%) which produced a strong deshielding of a signal to δ 4·84 and 4·97 (each 1H, *m*, $W_{1/2}$ 24 Hz), thus suggesting that the primary OH group at C-6″ is free. The ^{13}C NMR spectrum of (**6**) and its permethylated derivative (**7**), compared with that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside [4], also indicated the presence of a free primary hydroxyl at C-6″ in compound (**6**) (Table 1). This was confirmed by acid hydrolysis of (**6**) which yielded 2,3,4-tri-*O*-methylglucose. Hence, the full structure of lindleyin is **1**.



EXPERIMENTAL

Mp's, determined on a Kofler block, are uncorr. NMR spectra at 60 MHz were taken on a Perkin-Elmer R-12B instrument, those at 240 MHz on an IEF apparatus (Institut d'Electronique Fondamentale) and ^{13}C NMR spectra on a Bruker model HX90E, all with TMS as internal reference. Column and dry column chromatography was performed on Si gel 0·2–0·5 and 0·063–0·20 mm respectively.

Isolation process. Fresh whole plant (4·7 kg), collected at San Andrés (Tenerife) in June, was Soxhlet extracted with EtOH. Concentrated syrupy residue was extracted several times with EtOAc–MeOH; the combined solns were treated with $CHCl_3$ to eliminate labdanes, concentrated and residue percolated on Si gel (EtOAc–MeOH, 49:1), yielding lindleyin (3 g).

Lindleyin (**1**). mp 210–211° (MeOH–C_6H_6), $[\alpha]_D$ +14° (Py; *c* 0·38). (Found: C, 57·52; H, 5·50. $C_{23}H_{26}O_{11}$ requires: C, 57·74; H, 5·43%). UV λ_{max}^{EtOH} nm (log ε): 230 (4·38), 282 (4·33). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 3040, 1600, 1500 (phenyl ring), 1690 (C=O). MS (probe) 70 eV *m/e* (rel. int.): 460·1377 ($M^+ - H_2O$; 1), 170 ($C_7H_5O_6$; 44), 164 ($C_{10}H_{12}O_2$; 85), 153 ($C_7H_5O_4$; 92), 107 (C_7H_7O; 100), 94 (64), 91 (C_7H_7; 30), 43 (MeCO; 99).

Partial methylation of (**1**). A soln of (**1**) (1·0 g) in MeOH (200 ml) was treated with excess CH_2N_2, giving (**2**) (1·04 g), mp 145–147° (MeOH), $[\alpha]_D$ −85° ($CHCl_3$; *c* 0·10). (Found: C, 59·86; H, 6·25. $C_{26}H_{32}O_{11}$ requires: C, 60·00; H, 6·15%.) IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 3040, 1590, 1510 (phenyl ring), 1715 (C=O). MS (probe) 70 eV *m/e* (rel. int.): 520 M^+ (3), 357 (9), 356 (21), 221 (77), 195 (88), 164 (100), 107 (94), 94 (92), 91 (80), 43 (85). NMR (60 MHz, $CDCl_3$): δ 2·10 (3H, *s*, C-1), 2·70 (4H, *m*, $W_{1/2}$ 7 Hz, C-3, C-4), 3·85 (6H, *s*, Ph-O*Me*), 3·94 (3H, *s*, Ph-O*Me*), 6·68, 6·86, 7·14 (4H, A_2B_2, C-2′, C-3′, C-5′, C-6′).

Methanolysis of (**2**). (**2**) (0·5 g) was left overnight with satd methanolic Na_2CO_3 (150 ml) at room temp. Dry column chromatography (C_6H_6–EtOAc, 4:1) of the product gave tri-*O*-methylgallic acid methyl ester (210 mg) and (**3**) (260 mg). The former had mp 83–83·5° (subl) (Found: C, 58·30; H, 6·46. Calc. for $C_{11}H_{14}O_5$: C, 58·40; H, 6·24%.) and was identified by comparison with a sample prepared from gallic acid (mmp, TLC, IR, NMR).

Table 1. ^{13}C NMR data (22·63 MHz, CD_3CN) in δ units (TMS)

Compound	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
(**6**)	101·93	84·51	87·04	80·09	76·58	61·88
(**7**)	101·67	84·31	86·91	80·15	75·28	72·09
Methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside	105·00	84·58	87·21	80·48	75·38	72·36

Glucoside (3). mp 112–115° (EtOAc–$CHCl_3$). (Found: C, 58·84; H, 7·02. $C_{16}H_{22}O_7$ requires: C, 58·89; H, 6·79%.) IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 3050, 1610, 1510 (phenyl ring), 1710 (C=O). MS (probe) 70 eV *m/e* (rel. int.): 326 M^+ (0·5), 165 (32), 164 (100), 149 (24), 121 (28), 107 (60), 94 (60), 91 (20), 43 (36). *Tetraacetate* (4), prepared from (3) as usual, mp 129–131° (EtOAc–*n*-C_6H_{14}). (Found: C, 58·46; H, 6·24. $C_{24}H_{30}O_{11}$ requires: C, 58·30; H, 6·08%.) IR ν_{max}^{KBr} cm^{-1}: 3050, 1610, 1590, 1510 (phenyl ring), 1750, 1240 (OAc), 1712 (C=O). NMR (60 MHz, $CDCl_3$). δ 2·03 (9H, *s*, OAc), 2·08 (3H, *s*, OAc), 2·12 (3H, *s*, C-1), 2·78 (4H, *m*, $W_{1/2}$ 9 Hz, C-3, C-4), 3·88 (1H, *m*, $W_{1/2}$ 18 Hz, C-5″), 4·24 (2H, *m*, $W_{1/2}$ 8 Hz, C-6″), 5·17 (4H, *m*, $W_{1/2}$ 15 Hz, C-1″, C-2″, C-3″, C-4″), 6·82, 6·97, 7·05, 7·20 (4H, A_2B_2, C-2′, C-3′, C-5′, C-6′).

Acid hydrolysis of (3). A soln of (3) (0·2 g) in MeOH (8 ml) was refluxed with 2 N HCl (12 ml) for 2 hr. It was neutralized, concentrated and extracted with Et_2O. Syrupy residue (180 mg) was washed with hot MeOH, filtered and the solvent evaporated. PC on Whatman No. 1 in different systems showed the residue (30 mg) to consist of glucose. Concentration of the Et_2O extract gave 4-(*p*-hydroxyphenyl)-2-butanone (80 mg), mp 80–82° (*n*-C_6H_{14}–C_6H_6). (Found: C, 72·90; H, 7·43. Calc. for $C_{10}H_{12}O_2$: C, 73·14; H, 7·37%.) IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 3020, 1610, 1590, 1510 (phenyl ring), 1690 (C=O). NMR (60 MHz, $CDCl_3$): δ 2·12 (3H, *s*, C-1), 2·78 (4H, *m*, $W_{1/2}$ 7 Hz, C-3, C-4), 2·87, 3·01, 3·17, 3·31 (4H, A_2B_2, C-2′, C-3′, C-5′, C-6′). It was identified with the compound synthesized below (mmp, TLC, IR, NMR).

Synthesis of 4-(p-*hydroxyphenyl*)-2-*butanone*. To a soln of 3-(*p*-hydroxyphenyl)propionic acid (0·49 g, 3 mM) in Et_2O (20 ml) MeLi (7 mM) was added under N_2 at room temp. After refluxing for 30 min and cooling down H_2O and more Et_2O were added and the ethereal phase was concentrated *in vacuo*. Chromatography (C_6H_6–EtOAc, 9:1) yielded 4-(*p*-hydroxyphenyl)-2-butanone (0·15 g), mp 80–82° (*n*-C_6H_{14}–C_6H_6) (lit. [5] 82·5°).

Enzymatic hydrolysis of (3). β-Glucosidase (80 mg) was added to a soln of (3) (50 mg) in aq. 0·01 N NaOAc (20 ml) buffered with HOAc to pH 5, and allowed to stand at 37° under shaking for 16 hr. The soln was concentrated, extracted with Et_2O and the residue and Et_2O phase were treated as described for the acid hydrolysis of (3), giving glucose (6 mg) and 4-(*p*-hydroxyphenyl)-2-butanone (25 mg). The latter was identical with the compound synthesized above (mmp, TLC, IR, NMR).

Permethylation of (2). To a soln of (2) (1·0 g) in DMF (250 ml) MeI (2·5 ml) and Ag_2O (2·7 g) were added and the mixture stirred at room temp. for 20 hr. It was filtered, the ppt. was washed with DMF and the combined extracts were concentrated *in vacuo*, reextracted with $CHCl_3$ and filtered. Chromatography (C_6H_6–EtOAc, 9:1) of the $CHCl_3$ residue gave (5) (0·85 g) which would not crystallize. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2840, 1250 (OMe), 3020, 1610, 1580, 1500 (phenyl ring), 1730 (C=O). NMR (60 MHz, CCl_4): δ 2·00 (3H, *s*, C-1), 2·60 (4H, *m*, $W_{1/2}$ 8 Hz, C-3, C-4), 3·58 (6H, *s*, OMe), 3·78 (12H, *s*, OMe), 6·80 (4H, *s*, $W_{1/2}$ 4 Hz, C-2′, C-3′, C-5′, C-6′), 7·20 (2H, *s*, aromatic gallyl protons).

Methanolysis of (5). Permethylated lindleyin (5) (0·85 g) was treated with Na_2CO_3 in MeOH as indicated previously for (2), obtaining tri-*O*-methylgallic acid methyl ester (0·27 g) and (6) (0·35 g) which would not crystallize. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 3030, 1610, 1505 (phenyl ring), 2840, 1230 (OMe), 1715 (C=O). MS (probe) 70 eV *m/e* (rel. int.): 368 M^+ (4), 164 (100), 149 (7), 121 (14), 107 (43), 94 (20), 91 (8), 43 (35). NMR (240 MHz, $CDCl_3$): δ 2·10 (3H, *s*, C-1), 2·70, 2·73, 2·80, 2·83 (4H, *m*, C-3, C-4), 3·58 (3H, *s*, OMe), 3·65 (6H, *s*, OMe), 4·88 (1H, *d*, *J* 8 Hz, C-1″), 6·89, 6·92, 7·14, 7·17 (4H, A_2B_2, C-2′, C-3′, C-5′, C-6′). [240 MHz, $CDCl_3$ + 20% Eu(fod)$_3$]: δ 4·84, 4·97 (each 1H, *m*, $W_{1/2}$ 24 Hz, C-6″). ^{13}C NMR (22·63 MHz, CD_3CN): δ 29·58 (C-1 or C-4), 30·10 (C-4 or C-1), 45·57 (C-3), 60·65 and 60·78 (3 OMe), 117·40 (C-3′, C-5′), 130·33 (C-2′, C-6′), 136·57 (C-1′), 156·66 (C-4′), 208·99 (C-2); remaining signals see Table 1.

Permethylation of (6). (6) (80 mg) was permethylated as described above for (2), obtaining (7) (70 mg) which would not crystallize. IR: lacks OH absorptions. ^{13}C NMR (22·63 MHz, CD_3CN): δ 29·51 (C-1 or C-4), 30·10 (C-4 or C-1), 45·57 (C-3), 59·28 (OMe at C-6″), 60·65, 60·78 and 60·97 (3 OMe), 117·27 (C-3′, C-5′), 130·33 (C-2′, C-6′), 136·51 (C-1′), 156·53 (C-4′), 208·99 (C-2); remaining signals see Table 1.

Acid hydrolysis of (6). A soln of (6) (0·1 g) in MeOH (4 ml) was treated with 2 N HCl (6 ml) as mentioned above for the acid hydrolysis of (3). PC of the neutral soln on Whatman No. 3 in BuOH–HOAc–H_2O (4:1:5) separated 4-(*p*-hydroxyphenyl)-2-butanone (30 mg) and 2,3,4-tri-*O*-methylglucose (10 mg). The former was identified by comparison with the previously synthesized compound and the latter by comparison with an authentic sample (PC [6], IR, NMR).

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REFERENCES

1. Baker, A. J., Eglinton, G., González, A. G., Hamilton, R. J. and Raphael, R. A. (1962) *J. Chem. Soc.* 4705.
2. Tegner, C. (1952) *Acta Chem. Scand.* **6**, 782.
3. Kuhn, R., Trischmann, H. and Löw, I. (1955) *Angew. Chem.* **67**, 32.
4. Haverkamp, J., van Dongen, J. P. C. M. and Vliegenthart, J. F. G. (1973) *Tetrahedron* **29**, 3431.
5. Prasad, D. and Chaudhury, D. N. (1962) *J. Indian Chem. Soc.* **39**, 735.
6. Petek, F. (1965) *Bull. Soc. Chim. France* 263.