(with isotopic ion peaks at M + 1 and M + 2 in accord with 5. IR, UV and NMR spectra of **3–5** were identical with those of authentic samples.

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HYDROCARBONS, STEROLS AND FATTY ACIDS OF *LOBARIA PULMONARIA*

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Key Word Index—*Lobaria pulmonaria*; lichen; aliphatic hydrocarbons; ergosterol; fecosterol; fatty acids.

Plant. Lobaria pulmonaria (L) Hoffm. (Stictaceae). *Source.* The lichen, growing on chestnut tree bark, was collected in September near Gallicano (Lucca, Italy). *Previous work.* Arabitol [1], gyrophoric acid [2], stictinic acid [3], thelephoric acid [4], proteins [5], transaminases [6].

Present work. The dried material (1·3 kg) was extracted with light petrol for 40 hr and the residue obtained on evaporation (17·8 g) was worked-up in the usual way [7].

Constituents. Percentages of compounds with respect to the dried plant: aliphatic hydrocarbons, 0·05; ergosterol (mp 158–160°, $[\alpha]_D$ − 132°; acetate, mp 172–175°, $[\alpha]_D$ − 93·5°), 0·08; fecosterol [8] (mp 134–136°, $[\alpha]_D$ + 45·5°; acetate, mp 137–139°, $[\alpha]_D$ + 35·2°, M^+ 440), 0·09; fatty acids, 0·96. Relative amounts of aliphatic hydrocarbons (%, GLC): C_{25}, 7·5; C_{26}, 1·3; C_{27}, 21·5; C_{28}, 9·7; C_{29}, 32·6; C_{30}, 7·9; C_{31}, 19·5. Relative amounts of fatty acids (%, GLC of methyl esters): lauric, 0·2; tridecanoic, 0·6; myristic, 1·1; tetradecenoic, 0·5; pentadecanoic, 0·6; pentadecenoic, 1·5; palmitic, 51·3; palmitoleic, 0·3; heptadecanoic, 0·5; heptadecenoic, 0·2; stearic, 3·2; oleic, 20·4; linoleic, 13·5; linolenic + arachidic, 1·5; gadoleic, 2·5; behenic,2·0.

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A NEW COUMARIN, FRAXIDIN 8-*O*-β-D-GLUCOSIDE AND 10-HYDROXYLIGSTROSIDE FROM BARK OF *FRAXINUS EXELSIOR*

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Key Word Index—*Fraxinus exelsior*; Oleaceae; coumarins; fraxidin 8-*O*-β-D-glucoside; mandshurin; iridoid glucoside; 10-hydroxyligstroside.

Bark of the common ash, *Fraxinus exelsior* L., is known as a rich source of trioxygenated coumarins [1]; initially, the glucoside fraxin (**2**) [2,3] and the aglucones fraxidin (**3**), isofraxidin (**6**) and fraxinol (**9**) were identified,

the latter three isolated after hydrolysis of a mixture of non-characterized glycosides [4]. More recently, the glucoside of (6), calycanthoside (7) [5], was encountered in *F. exelsior* var. *diversifolia* Ait [6]. We report the isolation and characterization of fraxidin 8-*O*-β-D-glucopyranoside (4), from the bark of *F. exelsior*, along with some known compounds.

Extensive chromatographic fractionation (see Experimental) of a bark extract of *F. exelsior* afforded mandshurin (10), the glucoside of fraxinol (9), found so far only in *F. mandshurica* Rupr. var. *japonica* Maxim. [7] and characterized by comparison of its tetraacetate (11) with an authentic specimen. The mandshurin-containing fraction yielded an additional compound, isolated as a non-crystalline tetraacetate, isomeric with (11). The identity of the isomer as (5), rather than (8), suspected from ^{13}C NMR spectra [8], was corroborated by methylation of fraxin (2), followed by acetylation, to give a product that was indistinguishable from the tetraacetate of natural derivation.

The existence of an iridoid glucoside in the bark of *F. exelsior*, suspected from ^1H NMR signals observed on the crude extract, was confirmed by isolated of a non-crystalline iridoid glucoside hexaacetate. This compound possessed spectral characteristics confirming its identity as 10-hydroxyligstroside hexaacetate (13). The parent glucoside (12) has been reported from *Ligustrum obtusifolium* Sieb. et Zucc [9].

Iridoid glucosides, derived from oleoside [10] (14), have been encountered solely within the Oleaceae [11], known thus far from the genera *Fraxinus* [12,13], *Jasminum* [14], *Ligustrum* [9,15], *Olea* [16] and *Syringa* [13].

EXPERIMENTAL

Mps are corrected and determined in capillary tubes in a heated bath. Preparative TLC was performed on 20 × 40 cm plates coated with 1 mm layers of Si gel PF_{254} (Merck); bands were detected in UV light. ^1H NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard. Analyses were performed at NOVO Microanalytical Laboratories, Bagsværd, Denmark.

Extraction of bark. Fresh bark of *Fraxinus exelsior* (200 g, collected in October 1973 at Odden, Denmark) was homogenized in EtOH (1000 ml). After 4 days at 20°, the filtered extract was evaporated to nearly dryness. A soln of residue in H_2O (300 ml) was extracted with $CHCl_3$ (200 ml) and Et_2O (2 × 30 ml). The aq phase was concentrated and filtered through a column of Al_2O_3 (neutral, 100 g) in H_2O in order to remove tannins and flavonoids. After eluting the column with H_2O (500 ml), the combined eluates were mixed with Si gel (200 g) and solvent removed *in vacuo*. Powdery residue was placed at the top of a column of Si gel (packed in Me_2CO) and eluted with Me_2CO (2·5 l.). Evaporation of the solvent left an amorphous glycoside fraction (12·5 g).

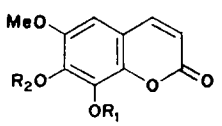
Fractionation of the glycoside mixture. An aliquot of the above mixture (4·3 g) in EtOH (75 ml) was left at 0° for 5 days, when it had deposited 320 mg of crystalline mannitol (mp 162–163°, mmp 161–164°). The mother liquors were concentrated and applied to a column of Si gel (325 g) in $CHCl_3$–MeOH (4:1) and eluted with the same solvent. Four fractions, A–D, each of 150 ml were collected. According to ^1H NMR spectra, *A* (335 mg) contained no coumarins and was discarded; *B* (1225 mg) contained what appeared to be the major coumarin glucoside, together with the same compounds as present in *A*. Fraction *C* (925 mg) contained an additional amount of the major coumarin, together with some minor coumarins and a compound exhibiting a sharp ^1H NMR signal at *ca* 7·5 ppm, suggestive of an iridoid (O–CH=C–COO) glucoside. Fraction *D* contained, besides minor coumarins, additional amounts of the latter compound. Repeated chromatography on columns and thick layer plates (Si gel), with $CHCl_3$–MeOH (4:1 and 3:1) and EtOAc–Bz–EtOH (4:1:1) as the eluents, and monitoring by ^1H NMR spectroscopy and TLC, resulted in separation of the major coumarin and the supposed iridoid glucoside into two fractions: E and F.

Coumarins. E (450 mg) was crystallized from EtOH to give mandshurin [(10); 130 mg], mp 177–178°; $[\alpha]_D^{22}$ −23° (*c* 0·9, MeOH). [Reported [7]: mp 182·5°; $[\alpha]_D^{22}$ −26·7° (*c* 3, MeOH)]. Acetylation (Ac_2O, Py) of the mother liquors, and chromatography of the product ($CHCl_3$–EtOAc–Et_2O; 1:1:1), gave two fractions. The faster running band contained pure mandshurin tetraacetate [(11); 325 mg] recrystallized from MeOH; mp 161–162·5°, undepressed on admixture with an authentic sample*; $[\alpha]_D^{22}$ −40° (*c* 0·5, EtOH). The slower moving band (81 mg), contained a homogeneous tetraacetate which could not be induced to crystallize. IR and ^1H NMR spectra of the latter compound were identical with those of a synthetic specimen (see below) of *fraxidin 8-O-β-D-glucoside tetraacetate* (5).

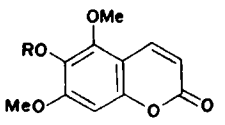
Iridoid. The syrupy fraction *F* (216 mg) was acetylated to give, after chromatography, amorphous hexaacetate (226 mg) of 10-hydroxyligstroside (13). IR and ^1H NMR spectra were identical with those recorded for an authentic specimen of 10-hydroxyligstroside hexaacetate (13). The UV spectrum of our isolate agreed well with that of the authentic, parent glucoside [(12); λ_{max}^{EtOH} 229 nm], whereas the reported [9] maximum (218 nm) for the hexaacetate was not observed in the case of our sample. Compounds with the same chromophore are reported [10] with maxima at 230–235 nm.

Fraxidin 8-O-β-D-glucoside (4). A suspension of fraxin (161 mg), isolated from bark of *Diervilla sessilifolia*, K_2CO_3 (500 mg), and Me_2SO_4 (0·35 ml) in Me_2CO (50 ml) was refluxed for 2 hr. The mixture was filtered, solvent evaporated, and residue chromatographed on plates with $CHCl_3$–MeOH (3:1) as the eluent. The main fraction, exhibiting a dark blue fluorescence in UV-light, yielded a syrup (140 mg, 84%), crystallizing from EtOH to give colourless crystals mp 193–195°; $[\alpha]_D^{19.5}$ −49° (*c* 0·5, H_2O); UV-spectrum: λ_{max}^{EtOH} 231 nm (ε 17·500), 295 nm (ε 9·600), and 343 nm (ε 6·200); addition of $AlCl_3$ did not change the spectrum. Found: C, 52·05; H, 5·29. $C_{17}H_{20}O_{10}$, 0·5 H_2O (393) requires: C, 51·93; H, 5·38%.

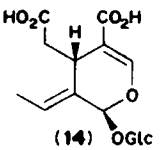
* The mp (85°) in ref. [7] has been reported to be in error [13]. [13].



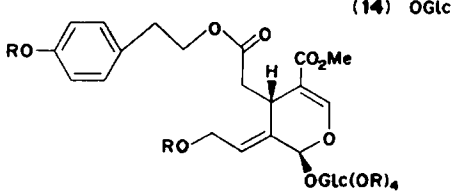
(1) $R_1 = R_2 = H$
(2) R_1 = Glc; $R_2 = H$
(3) $R_1 = H$; R_2 = Me
(4) R_1 = Glc; R_2 = Me
(5) R_1 = $GlcAc_4$; R_2 = Me
(6) R_1 = Me; $R_2 = H$
(7) R_1 = Me; R_2 = Glc
(8) R_1 = Me; R_2 = $GlcAc_4$



(9) R = H
(10) R = Glc
(11) R = $GlcAc_4$



(14) OGlc



(12) R = H
(13) R = Ac

Fraxidin 8-O-β-D-glucoside tetraacetate (**5**). Acetylation of (**4**) (50 mg), gave a colourless syrup (80 mg). The compound was purified by chromatography (Et_2O–EtOAc, 85:15), followed by passing a CH_2Cl_2-soln through charcoal and thorough drying; $[\alpha]_D^{22}$ −37° (*c* 0·4 in EtOH); UV-spectrum: λ_{max}^{EtOH} 230 nm (ε 17·800), 294 nm (ε 8·300), and 343 nm (ε 6·500). ^1H NMR spectrum: 7·60 and 6·33 ppm (*d*'*s*; $J_{2,3}$ 9·5 Hz, H-4 and H-3), 6·73 ppm (*s*; H-5), 5·50–5·10 ppm (*m*; H-1', H-2', H-3', H-4'), 4·21 and 4·13 ppm, (*dd*'*s*; H-6' and H-6''), 3·95 and 3·89 ppm (*s*'*s*; 2 × OMe), 3·75 ppm (*m*; H-5'), 2·11 and 2·00 ppm (1 and 3 OAc). Found: C, 54·15; H, 5·34, $C_{25}H_{28}O_{14}$ requires: C, 54·36; H, 5·11%.

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NORCAPILLENE, A NEW ACETYLENIC HYDROCARBON FROM THE ESSENTIAL OIL OF *ARTEMISIA CAPILLARIS**

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Key Word Index—*Artemisia capillaris*; Compositae; essential oil; acetylenic hydrocarbon; norcapillene.

In previous papers [1, 2], the structures of the new acetylenic compounds, 1-(2'-methoxyphenyl)-2,4-hexadiyne (*o*-methoxycapillene) and capillanol in the essential oil of *Artemisia capillaris* Thunb. have been described. We now report a new acetylenic hydrocarbon, norcapillene.

The compound constitutes *ca* 0·1% of the essential oil and was isolated by preparative GLC, using Celite 545 as the stationary phase. The compound analysed for $C_{11}H_8$, n_D^{25} 1·6364. IR spectrum shows –C≡C– str at 2220 and 2240 cm^{-1} (W), aromatic str at 1595 and 1490 cm^{-1} (M), aromatic adjacent 5H drf at 755 and 690 cm^{-1} (S). These data indicate that the compound is a aromatic monosubstituted hydrocarbon, with a C_5H_3 unit, whose structure $Ph(C{\equiv}C)_2Me$ (**1**) was elucidated from the NMR spectrum. This shows signals for 3 protons of methyl group in the α-position of the diacetylene bond at $\delta_{ppm}^{CCl_4}$ 1·98, as a singlet. The 5 protons in the benzene ring appeared as a broad singlet from δ 7·05 to 7·55. Consequently, the splitting pattern of the signals in the NMR spectrum appears to be in conformity with **1** for norcapillene. It had a UV spectrum almost superimposable with that of synthetic 1-phenyl-1,3-pentadiyne [3–6]. The formation of this phenylacetylene had been reported by H. Taniguchi *et al.* when 1-phenyl-1,4-pentadiyne and KOH in ethanol were kept under N_2 at *ca* 0° for 3 hr. The MS spectrum was also compatible with this structure. Besides the molecular ion peak at *m/e* 140 (98·2%) the other significant peaks discernible were at *m/e* 139 (M^+-H, 100·0%), 138 (M^+-H_2, 13·5%), 114 ($(\phi$–C≡C–C≡CH$)^+$, 29·8%), 113 (ϕ–C≡C–C≡C$^+$, 11·5%), 89 (7·2%), 88 (7·3%), 87 (10·1%), 63 (13·7%). Norcapillene was catalytically hydrogenated over PtO_2 in ethanol to give octahydronorcapillene, which was found to be identical with amylbenzene in all respects (IR, NMR, MS spectrum).

EXPERIMENTAL

Plant material and oil removal. A. capillaris was harvested in the suburbs of Osaka-Fu in October 1973. After steam distillation of 11·0 kg of the stalks and leaves, 88·3 g (0·803%) of the essential oil was obtained by the extraction of the distillate with Et_2O and by the evaporation of the solvent under N_2.

Isolation of norcapillene. Ten g of the essential oil was chromatographed on activated alumina (60 g, 300 mesh, a glass tube of *d* = 1·8 and *l* = 50 cm) with *n*-hexane to elute the terpene hydrocarbons. Subsequent elution with C_6H_6 gave norcapillene which was then isolated by prep. GLC (Carbowax-20 M 5%, 80–100 mesh, 4 mm 3·00 m, He 0·5 kg/cm^2).

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