Extraction. Leaves and stems were extracted with hot EtOH and conc *in vacuo*. Saccharose was separated by crystallization from the concentrate and the residue was chromatographed on Si gel and eluted with C_6H_6, C_6H_6–AcOEt, AcOEt, giving: diplopterol ($2.7 \times 10^{-3}\%$ of plant dry wt.), fernenol ($3.4 \times 10^{-3}\%$), sitosterol, the new natural triterpene 6*β*,22-dihydroxyhopane (**1a**, $4.8 \times 10^{-4}\%$) and sitosterol-*o* *β*-D-glucoside.

Diplopterol. Mp 234–236° (MeOH–Me_2CO), $[\alpha]_D + 42°$ (*c* 0.262). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH). PMR δ: 0.79 (3H, *s*, C-28), 0.84 (6H, *s*, C-23, C-24), 0.86 (3H, *s*, C-25), 0.98 (6H, *s*, C-26, C-27), 1.21 (6H, *s*, C-29, C-30). MS (70eV), *m/e* (rel. int.): 428 (M^+, 5), 207 (21), 191 (59), 189 (49), 149 (54), 121 (21), 95 (71), 59 (100).

Fernenol. Mp 198–200° (MeOH) $[\alpha]_D - 23°$ (*c* 0.216) [3]. *IR* ν_{max}^{KBr} cm^{-1}: 3610 (OH). PMR δ: 0.72, 0.76, 0.83, 0.88, 0.95, 1.06 (24H, eight Me groups), 3.15 (1H, *m*, $W_{1/2} = 18$ Hz, C-3), 5.35 (1H, *m*, $W_{1/2} = 10$ Hz, C-11). MS (70eV), *m/e* (rel. int.): 426 (M^+, 50), 411 (100), 393 (20), 273 (12), 255 (12), 259 (94), 241 (30), 95 (34). *Acetate*. Mp 218° (Me_2CO), $[\alpha]_D - 6°$ (*c* 0.246). (Found: C, 81.61; H, 11.15. Calc. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18%.) On chromic acid oxidation, fernenol furnished the corresponding *ketone*. Mp 197° (MeOH), $[\alpha]_D - 47°$ (*c* 0.254). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700 (CO). MS (15eV), *m/e* (rel. int.: 424 (M^+. 18), 410 (12), 409 (34), 271 (20) 258 (20), 257 (100), 246 (15), 245 (22). To our knowledge, this is the first reported instance of fernenol in ferns.

6β,22-Dihydroxyhopane. Mp 238–242° (MeOH), $[\alpha]_D + 19°$ (*c* 0.194). (Found: C, 81.09; H, 11.42. $C_{30}H_{52}O_2$ requires: C, 81.02; H, 11.79%.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3605 (OH). PMR δ: 0.79 (3H, *s*, C-28), 0.92 (3H, s, C-24), 0.96 (3H, *s*, C-27), 1.19 (3H, *s*, C-23), 1.21 (6H, *s*, C-25, C-26), 1.30 (6H, *s*, C-29, C-30), 4.55 (1H, *m*, $W_{1/2} = 10$ Hz, C-6). MS (70eV), *m/e* (rel. int.): 444 (M^+, 4), 426 (3), 411 (1), 207 (21), 191 (16), 189 (52), 149 (34), 59 (100). Attempts to acetylate and oxidize **1a** under mild conditions were unsuccessful, giving unchanged starting material.

6*β*, 22-*diacetoxyhopane* (**1b**). Acetylation of **1a** by refluxing for 30 min. with isopropenyl acetate and *p*-toluenesulfonic acid as catalyst gave (**1b**): mp 146–150° (MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1260 (OAc). PMR δ: 0.79 (3H, *s*, C-28), 0.94 (6H, *s*, C-24, C-27), 1.42 (3H, *s*, C-29 or C-30), 1.50 (3H, *s*, C-30 or C-29), 0.99, 1.15, 1.19 (9H, *s*, C-23, C-25, C-26 not respectively), 1.95 (3H, *s*, OAc), 2.02 (3H, *s*, OAc), 5.53 (1H, *m*, $W_{1/2} = 10$ Hz, C-6).

6β,22-Dihydroxyhopane (**1a**) from *zeorin* (**1d**). Zeorin was oxidized with CrO_3–AcOH–H_2O to zeorinone (**1c**) [4] which was reduced with $LiAlH_4$ to epizeorin (**1a**) [5], identical to the natural product (**1a**) (mmp, TLC, IR, PMR).

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6-METHOXYBENZOXAZOLINONE AND TRITERPENOIDS FROM ROOTS OF *SCOPARIA DULCIS*

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Key Word Index—*Scoparia dulcis*; Scrophulariaceae; 6-methoxybenzoxazolinone; betulinic acid; ifflaionic acid.

INTRODUCTION

Scoparia dulcis L. (Scrophulariaceae), known as a folk-medicinal plant, has been used as a cure for diabetes mellitus in India and for hypertension in Taiwan [1]. To date, only common constituents; sitosterol, hexacosanol, D-mannitol, tritriacontane [2,3] and some unidentified compounds: dulciol, scropanol, dulciolone [3] and an alkaloid [4] have been isolated from this plant. The biologically active substance has been found, although the Indian drug "amellin" [5], which was obtained from the fresh plant, has been used as the antidiabetic principle [6]. The present report deals with the isolation of three compounds; 6-methoxybenzoxazolinone, betulinic acid and ifflaionic acid from the plant material which was collected at Hsinchu, Taiwan and identified at the Herbarium of National Taiwan University.

RESULTS AND DISCUSSIONS

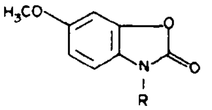
Dried powdered root of *Scoparia dulcis* was extracted with Et_2O. Subsequent separation of the Et_2O extract was achieved by repeated column chromatography using Si gel to give three crystalline compounds designated as compound A, B and C respectively.

Compound A (**1**), colourless needles from Me_2CO–petrol, mp 157–8°, IR ν_{max}cm^{-1}: 3350–3000 (N-H), 1770 (C=O, carbamate), 1620, 1490 (C=C, aromatic) and 1300 (C–N), UV λ_{max}nm (log ϵ): 209(4.01), 232(4.05) and 290 (3.80), had the molecular formula $C_8H_7NO_3$, as indicated by its molecular ion peak at *m/e* 165 and the elemental analysis. The NMR spectrum of compound A in $(CD_3)_2CO$ showed a three-protons singlet at 3.75 ppm corresponding to a methoxy group, and three aromatic protons at 6.62 (*dd*, $J_1 = 9$, $J_2 = 3$ Hz), 6.8 (*d*, $J = 3$ Hz) and 6.93 ppm (*d*, $J = 9$ Hz) indicating the presence of the 1,2,4-trisubstituted benzene ring. Compound A could be acetylated with Ac_2O–Py to give an acetyl derivative (**2**), mp 151–2° and methylated with CH_2N_2 to give a N-methyl derivative (**3**), mp 97–9°. More detailed NMR studies led to the structure of **1** as 6-methoxybenzoxazolinone which was isolated from maize and wheat plants [7]. This identification was confirmed by direct comparison (mmp, TLC, NMR and MS) with an authentic sample.

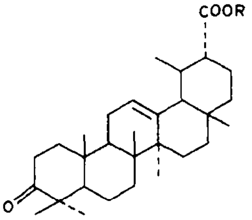
Compound B, colourless needles from MeOH, mp 290–2°, IR ν_{max}cm^{-1}: 3440 (OH), 1680 (COOH), 3060, 1620, 880 (C=CH$_2$), showed positive Liebermann–Burchard colour test and tetranitromethane test, and gave a monoacetate, mp 286–8°, IR ν_{max}cm^{-1}: 1720, 1235 (OAc), NMR (CDCl$_3$): δ 0.83 (9H, *s*, C-23, C-24 and C-25), 0.93 (3H, *s*, C-26), 0.97 (3H, *s*, C-27), 1.67 (3H, *s*, C-30), 2.00 (3H, *s*, OCOMe), 4.53, 4.66 (each 1H, *s*, C=C$\underline{H}_2$), 4.47 (1H, *m*, C$\underline{H}$-OAc). Treatment of the above acetate with CH$_2$N$_2$ yielded a monoacetyl methyl ester, mp 200–2°. Compound B and its above mentioned derivatives were then found to be identical in every respect (mmp, TLC, IR, and NMR) with betulinic acid, 3β-acetoxybetulinic acid and methyl-3β-acetoxybetulinate respectively.

Compound C (**4**), colourless needles from Me$_2$CO–*n*-hexane, mp 265–7°, C$_{30}$H$_{46}$O$_3$, positive Liebermann–Burchard, tetranitromethane and Zimmermann tests, IR ν_{max}cm^{-1}: 3230, 1735, 1690 (COOH), 1705 (C=O), UV λ_{max}nm (log ϵ): 205 (4.09), 271 (2.75), was also considered to be a triterpenoid. The NMR spectrum of compound C in CDCl$_3$ showed seven methyl groups at 0.83 (6H, *s*), 1.07, 1.10 (15H, each *s*), a complex signal at 2.47 (2H, *m*) assignable to two α-protons of carbonyl group and an incompletely resolved triplet corresponding to one olefinic proton centered at 5.03 ppm. Treatment of compound C with CH$_2$N$_2$ gave a methyl ester (**5**), mp 182–3°, IR ν_{max}cm^{-1}: 1730, 1695, whose NMR spectrum showed a three-protons singlet due to the carbomethoxy group at 3.65 ppm. On account of the above data, Compound C could be assigned as a ketonic acid of a pentacyclic triterpene having the ursane type skeleton. The MS of **5** was characterized by a molecular ion at *m/e* 468 and the base peak at *m/e* 262, indicating the presence of the carbomethoxy group in ring D or E, and a weak peak at *m/e* 247 (6% intensity of *m/e* 262) corresponding to loss of the C-17 methyl group from fragment-ion *m/e* 262, indicating that the carbomethoxy group must be placed at either C-19 or C-20 [8]. In conclusion, the methyl ester (**5**) was proved to be identical in all respects (mmp, TLC, IR, NMR and MS) with an authentic sample of methyl ifflaionate and compound C (**4**) was shown to be ifflaionic acid which has already been isolated from an Australian wood, *Flindersia ifflaiana* [9]. Ifflaionic acid is thus a rare naturally occurring triterpenoid.

It is significant to note that 6-methoxybenzoxazolinone has been shown to be a natural factor for the resistance of corn to European corn borer and to have a fungistatic effect to *Helminthosporium*, *Fusarium* and *Gibberella* infections [10,11]. Of particular interest to the present report was the demonstration that 6-methoxybenzoxazolinone has remarkable hypotensive activity by the primary animal tests. More detailed pharmacological studies are in progress to evaluate further the antihypertensive and antidiabetic potential of such compounds in *Scoparia dulcis*.



(1) R = H
(2) R = Ac
(3) R = Me



(4) R = H
(5) R = Me

EXPERIMENTAL

Mp's are uncorrected. UV spectra were determined in EtOH. IR spectra were recorded in KBr. NMR spectra were measured at 60 MHz with TMS as an internal standard. MS spectra were taken with a direct inlet system.

Extraction and isolation. Dried powdered root (1 kg) was extracted with Et$_2$O (3 l.) for 3 weeks. The Et$_2$O extract, after evaporation to dryness, yielded a residue (31 g). The residue was subjected to chromatography on a Si gel column. The fraction by elution with C$_6$H$_6$–Me$_2$CO (15:1) gave betulinic acid. On repeated Si gel column chromatography of the eluent from CHCl$_3$–EtOAc (9:1) gave 6-methoxybenzoxazolinone (**1**) and ifflaionic acid (**4**).

6-Methoxybenzoxazolinone (**1**). Colourless needles from Me$_2$CO–petrol (50–70°), mp 157–8°, M$^+$ 165. (Found: C, 58.57; H, 4.34; N, 8.54. Calc. for C$_8$H$_7$NO$_3$: C, 58.18; H, 4.27; N, 8.48%).

N-acetyl-6-methoxybenzoxazolinone (**2**). 50 mg of **1**, dissolved in 1.5 ml C$_5$H$_5$N, was treated at room temp. with Ac$_2$O (1.5 ml) for 12 hr and the reaction mixture treated in usual way. **2** was crystallized from Me$_2$CO–petrol, colourless needles, mp 151–2°, UV λ_{max}nm (log ϵ): 2.04 (4.38), 254 (3.89), 281 (2.87). IR ν_{max}cm^{-1}: 1720 (acetamide), 1770 (carbamate). NMR (CDCl$_3$): δ 2.73 (3H, *s*, N–CO–Me), 3.83 (3H, *s*, OMe), 6.72 (1H, *dd*, J_1 = 9 Hz, J_2 = 3 Hz), 6.83 (1H, *t*, J_1 = 3 Hz, J_2 = 3 Hz), 7.96 (1H, *dd*, J_1 = 9 Hz, J_2 = 3 Hz).

N-methyl-6-methoxybenzoxazolinone (**3**). **1** (25 mg) was allowed to stand overnight at room temp. with CH$_2$N$_2$ in Et$_2$O, and subsequent treatment in the usual way yielded **3**, colourless needles from CHCl$_3$–petrol, mp 97–9°, UV λ_{max}nm (log ϵ): 210 (3.64), 234 (3.78), 292 (3.52). IR ν_{max}cm^{-1}: 1265 (N-Me), 1280 (N-Ar), 1750 (carbamate). NMR (CDCl$_3$): δ 3.32 (3H, *s*, N-Me), 3.75 (3H, *s*, OMe), 6.70 (3H, *m*, aromatic).

Ifflaionic acid (**4**). Colourless needles from Me$_2$CO–*n*-hexane, mp 265–7°, M$^+$ 454. (Found: C, 79.31; H, 10.42. Calc. for C$_{30}$H$_{46}$O$_3$: C, 79.24; H, 10.20%).

Ifflaionic acid methyl ester (**5**). A soln of **4** (25 mg) in Et$_2$O was treated with ethereal CH$_2$N$_2$ at room temp. overnight and subsequent treatment in the usual way. **5** was crystallized from Me$_2$CO–MeOH, colourless needles, mp 182–3°.

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TRITERPENOIDS AND STEROLS OF *KALANCHOE SPATHULATA*

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Key Word Index—*Kalanchoe spathulata*; Crassulaceae; triterpenoids; freidelin; taraxerol; glutinol.

INTRODUCTION

Kalanchoe spathulata DC (Crassulaceae) is a herb distributed in the tropical Himalayas, Burma, China and Java [1]. The leaves of the plant are reported to be useful for the treatment of abscesses and of cholera [1], and the expressed juice is employed as an antiperiodic [2] and a drastic purgative [2, 3] in Indian indigenous medicine. Previous reports on the genus *Kalanchoe* indicated the presence of wax hydrocarbons, wax alcohols, sterols, triterpenoids [4, 5] phenolic compounds [6] and flavonoids [7] in *K. pinnata*. We now report the isolation and characterisation of long chain hydrocarbons and alkanols, sterols, and friedelin, glutinol and taraxerol (all triterpenoids) from the flowers of *K. spathulata*. This is the first report of the occurrence of such rearranged pentacyclic triterpenoids in the genus *Kalanchoe*.

RESULTS

Column chromatography on alumina of a petroleum ether extract of the air dried flowers of *K. spathulata* yielded a white wax, mp 63–64° (mixture of long chain hydrocarbon homologues by GLC) followed by a similar mixture of *n*-alkanols, mp 72–73°. On further elution three triterpenoids were obtained. Friedelin (D:A-friedooleanan-3-one), mp and mmp with an authentic sample 262–266°, NMR: δ 0.71, 0.86, 0.94, 1.04 and 1.18 (each 3H, *s*), 0.86 (3H, *d*, $J = 7$ Hz), and 1.00 (6H, *s*). Taraxerol (D-friedoolean-14-en-3β-ol) [8], mp 280–282°; acetate, mp 302–304°, NMR: δ 0.83, 0.87, 0.88, and 1.10 (each 3H, *s*), 0.91 and 0.96 (each 6H, *s*), 2.02 (3H, *s*, acetate), 4.45 (1H, *t*, $J = 7$ Hz), and 5.52 (1H, *dd*, $J = 3$ and 9 Hz). Glutinol (D:B-friedoolean-5-en-3β-ol), mp 208–210°, $[\alpha]_D + 61.5°$, NMR: δ 0.85, 0.94, 0.99, 1.00, 1.05, 1.09, 1.14 and 1.17 (each 3H, *s*), 3.46 (1H, broad *s*, $W_{1/2} = 7$ Hz) and 5.62 (1H, broad *s*, $W_{1/2} = 9$ Hz), MS (*m/e*): 426 (M^+) 408, 393, 274 and 259; acetate, mp 189–191°, $[\alpha]_D + 77°$, NMR: δ 0.85, 0.95, 0.99, 1.00, 1.05, 1.08, 1.10, 1.18 and 2.00 (each 3H, *s*), 4.70 (1H, broad *s*, $W_{1/2} = 6$ Hz), and 5.58 (1H, broad *s*, $W_{1/2} = 9$ Hz). The major peaks in the MS of glutinol and its acetate were those deriving from a *retro*-Diels–Alder type process in ring B analogous to that previously observed [9] in, for example, taraxerol. The final fractions from the column yielded a mixture of the plant sterols sitosterol (71%), stigmasterol (23%), and campesterol (6%).

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