Sweden. *Previous work.* Isolation of aromatin, aromaticin, mexicanin I and helenalin from *H. aromaticum* [1]. Isolation of helenalin from several species of the Compositae family [2], its structure [3]; stereostructure [4]; X-ray determination of bromohelenalin [5]. Isolation of linifolin A from *H. linifolium* Rydb. [6]; its structure [6]; stereostructure [7].

Present work. From the above-ground flowering part a lactonic fraction was isolated according to the previously described procedure [8]. The fraction gave, on chromatography on silica gel, linifolin A, mp 197–199°, $[\alpha]_D^{20}$ +30·4° (MeOH), $C_{17}H_{20}O_5$ (M^+ at *m/e* 304. Found: C, 66·87; H, 6·55. Calc: C, 67,09; H, 6·62%), IR ($CHCl_3$): 1760, 1712, 1661, 1154 cm^{-1}; CD (MeOH): $\Delta\epsilon_{338}$ −1·81; $\Delta\epsilon_{276}$ ±0; $\Delta\epsilon_{228}$ +26·07; $\Delta\epsilon_{218}$ ±0; $\Delta\epsilon_{208}$ −16·33, identical (mp, IR, $[\alpha]_D$, MS and PMR) with the described data of linifolin A [7]. From further fractions helenalin was isolated, mp 159–161°, $[\alpha]_D^{20}$ −70·0° (MeOH), $C_{15}H_{18}O_4$ (M^+ at *m/e* 262. Found: C, 68·41; H, 7·09. Calc: C, 68·68; H, 6·92%) IR ($CHCl_3$): 3610, 3500, 1764, 1705, 1658, 1156 cm^{-1}. CD (MeOH): $\Delta\epsilon_{326}$ −1·80; $\Delta\epsilon_{283}$ −0·44; $\Delta\epsilon_{240}$ −3·02; $\Delta\epsilon_{231}$ ±0; $\Delta\epsilon_{218}$ +4·31, identical (mp, mmp, IR, $[\alpha]_D$, MS and PMR) with an authentic sample.

Some South-American species of *Helenium* differ from North-American representatives of this genus only by minor morphological characters of the flower heads, but not always in their chemical components. It is of interest, therefore, that the detected linifolin A and helenalin are characteristic of *H. linifolium* Rydb. [6] from Texas and other North-American species [2]. It is also noteworthy that mexicanin I, aromatin and aromaticin which have been isolated from *H. aromaticum* of Chilean origin [1] were not detected in the plants of the same species cultivated in Poland. (Sheets of *H. aromaticum* from Poland were compared with those of *H. aromaticum* of Chilean origin which are deposited in the Herbarium of the National Museum in Prague and were found identical in all respects.) However, the separation of South-American *Helenium* taxa to an independent genus, *Cephalophora* Cav. [e.g. *Cephalophora aromatica* (Hook) Schrader] does not seem to be substantiated. On the contrary, the incorporation of *H. aromaticum* in the section Cephalophora (Cav.) Hoffmann, seems sufficient to us for the accentuation of the slightly different organisation of the flower heads.

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TRIGALACTOSYL DIGLYCERIDE OF PUMPKIN

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Key Word Index—*Cucurbita maxima*; Cucurbitaceae; pumpkin; trigalactosyl diglyceride; glycolipid.

Monogalactosyl diglyceride, digalactosyl diglyceride, sulfoquinovosyl diglyceride and trigalactosyl diglyceride (TGD) are known to be the main glyceroglycolipids occurring in plant kingdom. Although structures of the former three were well clarified [1, 2], that of TGD has not conclusively

been established yet. The present report describes the isolation, identification and chemical structure of TGD in pumpkin.

The glycolipid fraction from pumpkin gave a single spot (R_f 0·50) on TLC with $CHCl_3$–MeOH–H_2O (65:25:4) on silica gel G. The similarity of the IR spectrum of the lipid to those [3–5] of monogalactosyl diglyceride and digalactosyl diglyceride together with the strong absorption at 1070 cm^{-1} for alcoholic C–O resulting from sugar suggested that the lipid was a higher homologue of glyceroglycolipids. The molar ratio of glycerol [6] (6·8%), ester [7] (48·0%) and sugar [8] (45·2%) in the lipid was quite close to calculated values for linolenyl–linoleyl-trigalactosyl–glycerol (1:2:3), TGD.

The TGD was saponified and the component fatty acids were analyzed by GLC. Linolenic (60·0%) and linoleic (26·7%) acids were predominant. Webster and Chang [9] reported that spinach TGD was rich in linolenic acid. When the methyl glycosides obtained by methanolysis of deacylated TGD were analyzed by GLC, galactose was found to be the predominant component, as found for TGD in spinach [9] and potato [10]. Partial hydrolysates of deacylated TGD gave four spots by PC, corresponding to glycerol, galactose, monogalactosyl glycerol and digalactosyl (DG) glycerol, indicating that the deacylated lipid was trigalactosyl (TG) glycerol, with a third galactose linked to the terminal galactose moiety of DG-glycerol.

Treatment of TG-glycerol with α-galactosidase produced galactose and DG-glycerol, whereas β-galactosidase had no effect showing the terminal galactose unit of TGD had the α-configuration. When methyl glycosides obtained from ethanolysis of methylated TGD were analyzed by GLC, peaks for methyl-2,3,4,6-tetramethylgalactoside and methyl-2,3,4-trimethylgalactoside were the major products, with trace amounts of methyl-2,3,6-trimethylgalactoside. These results together with information in the literature [3, 11, 12], shows that TGD from pumpkin is 1,2-diacyl-3-*O*-[α-D-galactopyranosyl-(1′→6′)-*O*-α-D-galactopyranosyl-(1′→6′)-*O*-β-D-galactopyranosyl]-*sn*-glycerol.

EXPERIMENTAL

Isolation of TGD. Matured mesocarp of fresh pumpkin (*Cucurbita maxima*, var. Ebisu) was homogenized and extracted with $CHCl_3$–MeOH (2:1) [13] to obtain total lipid (0·88%). This was chromatographed on silicic acid column [14] with $CHCl_3$, Me_2CO and MeOH to be divided into fractions of neutral lipids, glycolipids and phospholipids. Crude TGD, obtained from rechromatography of the glycolipid fraction with $CHCl_3$–Me_2CO [15], was purified by preparative TLC [16].

Deacylation of TGD. TGD was treated with 0·4 N NaOH at 37° for 2 hr [17]. The mixture was acidified (pH 2·0) and extracted with hexane. The extracts were hydrolyzed with 5% HCl in MeOH and the resulting fatty acid methyl esters, were analyzed by GLC [18]. The residual phase after extraction was passed through column of Dowex-2 (OH^- form) and Dowex-50 (H^+ form) to obtain deacylated TGD.

Methanolysis of deacylated TGD. Deacylated TGD was refluxed with 5% HCl in MeOH for 4 hr. The products were purified by ion-exchange resin as described above to obtain the methyl glycosides, which were analyzed by GLC [19].

Partial hydrolysis of deacylated TGD. Deacylated TGD was hydrolyzed with 0·025 N HCl [20]. The partial hydrolysates were purified by passing through the ion-exchange columns and applied to PC with *n*-BuOH–pyridine–H_2O (6:4:3) [21].

Enzymatic hydrolysis of deacylated TGD. Deacylated TGD was treated with α-galactosidase prepared from coffee beans (the gift from Sapporo Medical College) or β-galactosidase (Sigma). The reaction was stopped by boiling, centrifuged and the supernatant was concentrated and applied to PC for analysis of sugar [21].

Methylation of TGD and its hydrolysis. TGD was fully methylated [22], methanolyzed with 5% HCl in MeOH and extracted with hexane. The residual soln was deionized by the ion-exchange columns and analyzed by GLC for methylated sugars [23].

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A NEW PENTACYCLIC TRITERPENE LACTONE FROM *DILLENIA INDICA**

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Key Word Index—*Dillenia indica*; Dilleniaceae; myricetin; sitosterol; betulinic acid; betulinaldehyde; betulin; lupeol; a new hydroxylactone—3β-hydroxy-lupane-13β, 28-lactone.

Plant. Dillenia indica Linn is an evergreen tree native to India. *Uses.* Its bark and leaves are astringent [1]. *Previous work.* On trunk bark [2], on leaves [3].

Present work. Air-dried powdered stem-bark was successively extracted with light petrol, $CHCl_3$ and MeOH. Light petrol extract was dried and separated into neutral and acidic fractions by the usual method. The neutral fraction on repeated chromatography on SiO_2 column afforded betulinaldehyde, m.p. 199–200° $[\alpha]_D^{26}$ + 28°,† betulin, m.p. 254–255° $[\alpha]_D^{27}$ + 22°, Lupeol, m.p. 211–212° $[\alpha]_D^{29}$ + 27°, sitosterol, m.p. 137° $[\alpha]_D^{28}$ − 36° and the acidic fraction yielded only betulinic acid, m.p. 305–306° $[\alpha]_D^{27}$ + 6·35°. These compounds were identified by comparison with authentic samples (m.m.p., co-TLC, superimposable IR). The isolation of betulinic acid, betulinaldehyde, betulin and lupeol showed a remarkable biogenetic sequence rarely encountered in a plant source. The $CHCl_3$ extract yielded only betulinic acid and betulin.

The dried MeOH extract was re-extracted with $CHCl_3$ to remove nonglycosides and the mixture of glycosides, which could not be separated on SiO_2, was hydrolysed with 5% methanolic HCl. The only sugar identified was D-glucose (PC). The aglycone fraction was separated into acidic and neutral parts. The acidic part yielded only betulinic acid, and the neutral part, on column chromatography on SiO_2, afforded sitosterol, betulin, hydroxylactone B of betulinic acid [4] along with the flavonol myricetin, m.p. 358–360°, acetate, m.p. 214–216° and identified by comparison with an authentic sample (m.m.p., Co-TLC, superimposable IR, UV). Hydroxylactone B was most probably an artefact formed by influence of acid on betulinic acid.

Besides this series of known compounds C_6H_6:$CHCl_3$ (4:1) eluate from the chromatogram yielded a triterpene (+ve Liebermann–Burchard test −ve, $C(NO_3)_4$) m.p. 325° (*d*), $[\alpha]_D^{27}$ + 63·4°. $C_{30}H_{48}O_3$ (Found: C, 78·45; H, 10·42%; Calc. for $C_{30}H_{48}O_3$, C, 78·89; H, 10·59%) MW 456 by MS. Acetate m.p. 319–320° $[\alpha]_D^{27}$ + 82·1°. IR, 1754 cm^{-1} (5-membered lactone) and 3350 cm^{-1} (hydroxyl), suggestive of a hydroxylactone. This lactone was different from hydroxylactone A and B by comparison with authentic samples [4].

On oxidation with Jone's reagent it gave a keto compound, m.p. 328–330° $[\alpha]_D^{27}$ + 70°; IR, 1689 and 1754 cm^{-1}. The ketone gave a positive Zimmerman's test and was reduced back to the original compound with $NaBH_4$ which proved the β conformation of OH group at C-3. Studies of its MS pattern [5] (prominent peak for M − 43 unit for *iso*propyl) NMR spectra [6] and also on biogenetic ground, indicated the compound to be in the lupane series. It was not identical with dihydrothurberogenin [7] or the dihydrolactone produced by mercuric acetate oxidation of acetyl betulinic acid [8].

The compound was reduced by $LiAlH_4$ to give a triol m.p. 280–281° $[\alpha]_D^{27}$ + 34°, (Found: C,

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† Unless otherwise stated, all $[\alpha]_D$ values are in $CHCl_3$.