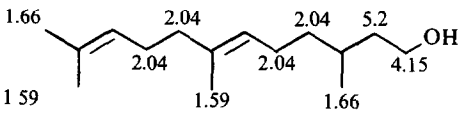

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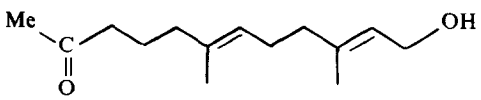

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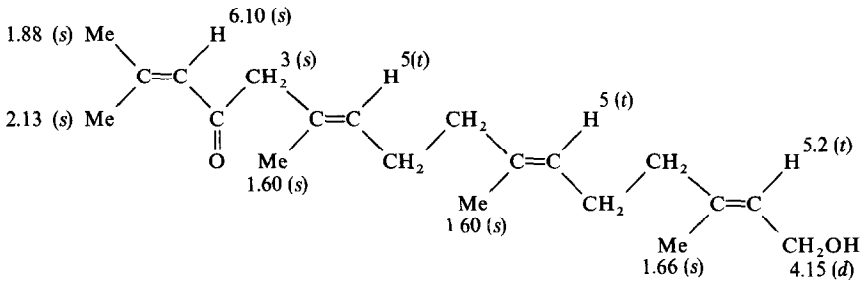

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LUPA-20, 29-ENE-3-*O*-β-D-MALTOSIDE FROM THE ROOTS OF *CORDIA OBLIQUA*

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Key Word Index—*Cordia obliqua*; Boraginaceae; triterpene glycoside; lupa-20,29-ene-3-*O*-β-D-maltoside.

INTRODUCTION

Cordia obliqua (Boraginaceae) is reputed for its medicinal importance [1, 2]. However little work appears to have been reported on the roots of this plant.

RESULTS AND DISCUSSION

A new triterpene-glycoside, mp 186–187° (dec.) was isolated from the $CHCl_3$ soluble fraction of the white crystalline deposit obtained from the EtOH extract of the roots of *Cordia obliqua*. The glycoside gave characteristic properties of the saponins. It gave a copious foam when shaken with water, haemolysed red blood cells and was toxic to fish. Hydrolysis (7% ethanolic H_2SO_4) of the glycoside yielded a white aglycone $C_{30}H_{50}O$, mp 212–213°; $[\alpha]_D^{25}$ + 28°; (in $CHCl_3$) and a sugar which was identified as glucose by its cochromatography with an authentic sample.

From the detailed study of the MS, IR and NMR spectra the aglycone was shown to be lupeol which was confirmed by mp, mmp and cochromatography with an authentic sample.

Periodate oxidation consumed 3.12 moles of periodate with the production of 1.01 moles of formic acid per mole of the glycoside, suggesting the presence of two units of monosaccharide in the pyranose form of the sugar. Acid hydrolysis of the completely methylated glycoside afforded two products, which were identified as 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose by cochromatography with authentic samples. The sugar moiety was found to be present as maltose which was confirmed by cochromatography with an authentic sample.

The glycoside could be hydrolysed with emulsim to yield the aglycone and maltose thus indicating the linkage between maltose and the aglycone as β- and α-linkage between the two units of glucose. Had the two units of glucose been joined by a β-linkage, emulsin hydrolysis would have given glucose and not maltose. With regard to the configuration of the saponin–glycoside linkage, it is a general observation that D-sugars occur with a β-linkage and L-sugars with an α-linkage [3].

On the basis of this study structure **1** has been assigned to the glycoside.

EXPERIMENTAL

Isolation and purification. The air dried, powdered roots of *Cordia obliqua*, procured from the United chemicals and allied products, Calcutta (India), were extracted with hot EtOH for 120 hr under reflux. The extract was filtered hot and while kept in a refrigerator for a few days deposited a white crystalline compound. The filtrate was concd to half vol. and again kept in a refrigerator for a few days. A similar white crystalline compound was obtained. These were combined and defatted with petrol (bp 40–60°). The petrol insoluble portion was then extracted with C_6H_6 and $CHCl_3$. The $CHCl_3$ soluble fraction gave the glycoside which was crystallized as a pure compound from $CHCl_3$–MeOH mixture. It was homogeneous on TLC ($CHCl_3$–MeOH, 9:1; $CHCl_3$–MeOH, 4:1). (Found C, 66.98;

H, 9.31; Calc. for $C_{42}H_{70}O_{11}$: C, 67.20; H, 9.33%). Allantoin was obtained from the $CHCl_3$ insoluble fraction.

Acid hydrolysis of the glycoside. 200 mg of the glycoside were hydrolysed with 50 ml 7% ethanolic H_2SO_4 for 4 hr at 100°. The hydrolysed product was poured into excess of H_2O. On cooling a solid ppt. was obtained which was washed with H_2O and crystallized as white silky needles from hot EtOH, mp 212–213°. It gave all the characteristic tests for triterpenoids [4] (Found: C, 84.36; H, 11.69; Calc. for $C_{30}H_{50}O$, C, 84.50; H, 11.73%). It was identical with lupeol by mp, mmp, IR, NMR and MS [5, 6].

Acetylation and acetyl percentage determination. 50 mg of the aglycone were acetylated (Ac_2O–Py) and the acetyl derivative was crystallized from MeOH mp 213–214°, $[\alpha]_D^{25}$ + 47 00 (in $CHCl_3$). The percentage of acetyl group was determined [7, 8]. Found. COMe, 8.98%; Calc. for $C_{30}H_{49}O$ (COMe), 9.18%.

Oxidation of the aglycone with chromic acid [9]. A cold soln of chromic acid (2.67 g) in conc H_2SO_4 (50 ml) and H_2O (10 ml) was made up to 100 ml. The aglycone was dissolved in Me_2CO and the reagent added dropwise until a persistent orange-brown coloration was obtained. The separated product was recrystallized from Me_2CO, mp 168°.

Methylation of the glycoside. A mixture containing 100 mg of the glycoside in 25 ml dry Me_2CO, 4 g dry K_2CO_3 and 5 ml Me_2SO_4 was heated for 8 hr at 100°. After distilling off Me_2CO under red. press., H_2O was added to decompose remaining Me_2SO_4. The compound obtained on filtration was washed well with H_2O and dried *in vacuo*. To ensure complete methylation it was further methylated as in [10]. The methylated product was crystallized from MeOH. The permethylated product was dissolved in MeOH (25 ml) and hydrolysed under reflux for 4 hr with conc HCl. The MeOH was evapd at 100 keeping the total vol constant by gradual addition of H_2O. The pptd aglycone filtered The filtrate was neutralized with Ag_2CO_3. Pptd AgCl was filtered and the filtrate evapd to a syrup. The methylated sugars in the syrup when resolved by PLC (BuOH–H_2O, azeotrope, spraying reagent aniline hydrogen phthalate) gave two spots corresponding to 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose. The respective zones were eluted with H_2O and evapd *in vacuo* to a syrup. 2,3,6-tri-*O*-methyl-D-glucose had $[\alpha]_D$ + 67° (in H_2O). It was identified by preparing its *p*-nitrobenzoyl derivative [11], and comparison with an authentic sample (mp and mmp 190–192°). 2,3,4,6-Tetra-*O*-methyl-D-glucose had $[\alpha]_D$ + 83.1° (in H_2O) and was confirmed by cochromatography with an authentic sample.

Hydrolysis of the glycoside with formic acid. 30 mg of the glycoside were dissolved in boiling C_6H_6 and 7% soln of HCOOH (8 ml) was added. The reaction mixture was refluxed for 30 min, solvent distilled off and the conc reaction mixture was poured into excess H_2O. The lupeol ppt. was filtered and the aq. hydrolysate identified as maltose (PC, *n*-BuOH–HOAc–H_2O, 4:1:5; spray reagent: aniline hydrogen phthalate). This sugar on further hydrolysis with 7% aq. H_2SO_4, gave only glucose.

Enzymatic hydrolysis of the glycoside [12, 13]. The glycoside (20 mg) was hydrolysed with emulsin at 40–50° for 12 hr. Liberation of maltose was detected by PC.

Periodate oxidation of the glycoside. 30 mg glycoside dissolved in 25% EtOH were treated with 25 ml 0.1 M Na metaperiodate. The periodate consumed and formic acid liberated were estimated [14]. For each mole of the glycoside, 3.12 moles of periodate consumed and 1.01 moles of formic acid liberated.

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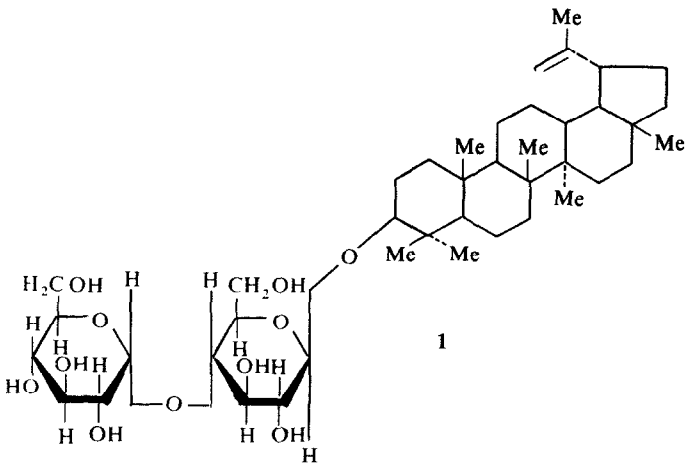

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