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TWO RHAMNETIN DIGALACTOSIDES AND AN OLEANOLIC ACID DIGALACTOSIDE FROM THE FLOWERS OF *CASSIA LAEVIGATA*

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Key Word Index—*Cassia laevigata*; Leguminosae; flowers; rhamnetin 3-galactosyl(1→4)galactoside; rhamnetin 3-galactosyl(1→6)galactoside; oleanolic acid 3-galactosyl(1→4)galactoside.

Abstract—From the flowers of *Cassia laevigata*, two new rhamnetin glycosides, the 3-galactosyl(1→4)-galactopyranoside and the related 3-galactosyl(1→6)galactopyranoside, and oleanolic acid 3-galactosyl(1→4)-galactopyranoside have been isolated. These three glycosides have not been isolated earlier from any plant source. The known compounds quercetin, docosyl alcohol, carnaubyl alcohol, ceryl alcohol and octacosanol have also been obtained.

INTRODUCTION

Species of *Cassia* are rich sources of flavonoids, anthraquinones and polysaccharides. Flavonoids[1, 2] and anthraquinones[3] have previously been isolated from *Cassia laevigata*. The plant possesses important medicinal properties [4].

RESULTS AND DISCUSSION

From the flowers of *Cassia laevigata* two flavonol glycosides (**1**) $C_{24}H_{32}O_{17}$ mp 215°(d), (**2**) $C_{24}H_{32}O_{17}$ mp 271°(d) and a triterpene-carboxylic acid glycoside (**3**) $C_{42}H_{68}O_{13}$ mp > 300°, and the known compounds quercetin docosyl alcohol carnaubyl alcohol, ceryl alcohol and octacosanol have been isolated.

1 on acid hydrolysis gave an aglycone and galactose. The aglycone $C_{16}H_{12}O_7$, mp 284°gave all characteristic colour reactions of a flavonol[5] and was identified as rhamnetin on the basis of UV, IR, NMR, mass fragmentation pattern, chemical degradation and co-chromatography with an authentic sample.

Methylation of **1** followed by acid hydrolysis gave 5, 7, 3′, 4′-tetramethylquercetin confirming the attachment of a sugar moiety at position-3. The glycoside was fully methylated, hydrolysed and the resulting partially methylated sugars were identified as 2, 3, 6-tri-*O*-methylgalactose and 2, 3, 4, 6-tetra-*O*-methylgalactose[6, 7]. This established the glycoside as a (1→4) bioside of galactose linked at position-3 of the aglycone. This was also confirmed by periodate oxidation. The glycoside was completely hydrolysed by β-glucosidase showing the presence of two β-linkages. On this basis **1** was identified as rhamnetin 3-*O*-β-D-galactosyl(1 → 4)-*O*-β-D-galactopyranoside. This glycoside has not been isolated earlier from any plant source.

2 was also a flavonol glycoside and on acid hydrolysis again gave rhamnetin and galactose. The attachment of the sugar moiety at position-3 was established as above. The permethylated glycoside gave 2, 3, 4-tri-*O*-methylgalactose and 2, 3, 4, 6-tetra-*O*-methylgalactose on hydrolysis with 4 N sulphuric acid which established that **2** is a (1→6) bioside linked at position-3 of the aglycone. This was also confirmed by periodate oxidation. The glycoside was completely hydrolysed with emulsin thereby showing the presence of β-linkages. On the above facts **2** was identified as rhamnetin 3-*O*-β-D-galactosyl(1→6) galactopyranoside. This glycoside is also new to nature.

3 was a terpene glycoside and on acid hydrolysis gave an aglycone and galactose. The aglycone, $C_{30}H_{48}O_3$, mp 306°, responded to all the colour tests for a triterpenoid and its IR spectrum showed the presence of a hydroxyl group, an acid carbonyl, a gem

dimethyl group, and a trisubstituted double bond. This evidence suggests that the aglycone is a triterpenic acid. Both a monomethyl ether and a monoacetate were formed. The ^{1}H NMR spectrum of the methyl ester acetate confirmed the presence of one double bond (δ 5.30), an acetoxyl group (δ 2.05), a carbomethoxyl (δ 3.64) and seven methyl groups [8] and its mass fragmentation pattern (*m/z* 262, 249, 203 and 133) indicated the presence of a C_{12}–C_{13} double bond, a C_{17} carbomethoxy and an acetyl group at C_3 in the molecule [9]. All the above results clearly indicated that the aglycone is oleanolic acid which was also confirmed by mp, mmp and co-chromatography with an authentic sample.

The glycoside (**3**) was methylated with diazomethane and the IR spectrum of the methyl ether showed a peak at 1739 cm^{-1}. The formation of a methyl ether of the glycoside confirmed that galactose is attached to a hydroxyl group not to a carboxyl group. This has also been confirmed by facile hydrolysis of the glycoside with acid but not with alkali. The permethylated glycoside on acid hydrolysis gave 2, 3, 4, 6-tetra-*O*-methylgalactose. This confirmed that **3** is a (1→4) bioside of galactose. The glycoside was completely hydrolysed with emulsin thereby showing the presence of β-linkages. Hence **3** is oleanolic acid 3-*O*-β-D-galactosyl(1→4)-*O*-β-D-galactopyranoside. This glycoside is also new.

The identification of quercetin, docosyl alcohol, carbonyl alcohol, ceryl alcohol and octacesanol were confirmed by mp, mmp and co-chromatography with authentic samples. These compounds have been isolated from many *Cassia* species [1, 2, 10, 11].

EXPERIMENTAL

The flowers of *Cassia laevigata* were obtained from United Chemicals and Allied Products, Calcutta-1, India. The flowers were extracted with ethanol and the concentrated extract diluted with water to give an aq. soln (Fraction 1) and coloured residue (Fraction 2).

Fraction 1. The aq. fraction was concentrated and successively extracted with ether and ethyl acetate. Extraction with Et_2O and chromatography over silica gel with benzene–EtOAc (3:2) gave **1** mp 215°(d) and extraction with EtOAc and chromatography over silica gel with EtOAc gave **2**, mp 271°(d). Further elution with EtOH gave (**3**) mp > 300°(d).

Rhamnetin 3-galactosyl(1→4)galactoside (**1**). UV λ_{max}^{EtOH} nm 255, 355; $AlCl_3$ 260, 405, $AlCl_3$/HCl 265, 402; NaOAc 255; NaOAc/H_3BO_3 260, 389. ^{1}H NMR (90 Hz) $(CD_3)_2SO_4$: δ 3.80 (Me, 3H, *s*) 6.20 (d, 1H, H-60), 6.45 (*d*, *J* = 2.5 Hz, 1H, H-8) 690 (*d*, *J* = 8.5 Hz, 1H, H-5′), 7.60 (*t*, *J* = 8.5, 2.5, 2H, H-6′, H-2′), 3.54 (10-H, sugar protons) and 5.65 (H-1 galactosyl).

On hydrolysis **1** gave rhamnetin and galactose (PC in EtOAc–pyridine–H_2O; 12:5:4 and EtOAc–PrOH–H_2O; 3:1:1). Methylation (Me_2SO_4–K_2CO_3) and acid hydrolysis gave 5, 7, 3′, 4′-tetramethylquercetin, identified by mmp and co-chromatography with an authentic sample. **1** was methylated with Me_2SO_4 in aq. NaOH and the methylated glycoside, mp 140°(d) hydrolysed with 4 N H_2SO_4. The partially methylated sugars were identified by PC in *n*-BuOH–EtOH–H_2O (5:1:4). Methylated galactose were identified by the methods of refs. [5, 6].

Rhamnetin 3-galactosyl(1→6)galactoside (**2**). UV λ_{max}^{EtOH} 255, 356, $AlCl_3$ 270, 407; $AlCl_3$/HCl 265, 400; NaOAc 256, NaOAc/H_3BO_3 262, 390 nm. ^{1}H NMR (90 Hz), $(CD_3)_2SO_4$: δ 3.80 (*s*, 3H, –OMe), 6.22 (*d*, 1H, H-6), 6.45 (*d*, *J* = 2.5 Hz, 1H, H-8), 6.92 (*d*, *J* = 8.5 Hz, 1H, H-5′), 7.60 (*t*, *J* = 8.5, 2.5 Hz, 2H, H-6′, H-2′), 3.56 (1OH sugar protons) and 5.67 (H-1 galactosyl).

Oleanolic acid 3-galactosyl(1→4)galactoside (**3**). UV λ_{max}^{EtOH} 236 nm. Acetate (Ac_2O/pyridine) mp 160°.

Isolation of aglycone. **3**, in EtOH was refluxed for 24 hr in 7% H_2SO_4. The ppt was filtered, dissolved in Et_2O, washed with H_2O and dried (Na_2SO_4) mp 306°. IR ν_{max}^{KBr} 3452, 1686, 1365, 815 cm^{-1}. Monomethyl ether (diazomethane) mp 196°, Acetate (Ac_2O/pyridine) mp 268°, methyl ester acetate, mp 225°, ^{1}H NMR: δ 5.30 (C=C), 2.05 (MeCO), 3.64 (carbomethoxy) and 0.18–1.12 (CH_2), MS *m/z* 262, 249, 203, 133.

Fraction 2. Extraction with EtOAc yielded quercetin mp 316°.

Isolation of aliphatic alcohols. An EtOH extract of the flowers of *Cassia laevigata* was hydrolysed with 1 N NaOH and the unsaponifiable material extracted with benzene and chromatographed over alumina and eluted with different mixtures according to their polarity. Petrol yielded docosyl alcohol; petrol–benzene (4:1) yielded carnaubyl alcohol; petrol–benzene (1:1) yielded ceryl alcohol; and benzene (100%) yielded octacosanol. These compounds were identified by mp, mmp, IR, NMR spectra and mass fragmentation patterns.

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