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A NEW GLYCOSIDE, ACACETIN-7-GLUCURONO-(1→2)-GLUCURONIDE FROM THE LEAVES OF CLERODENDRON TRICHOTOMUM

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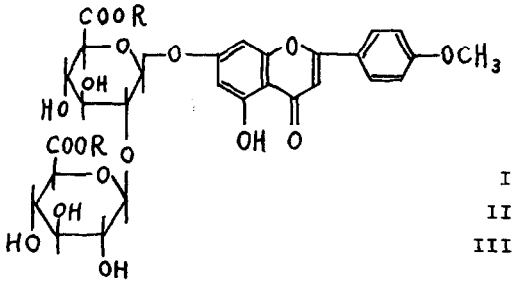
Glycyrrhizin[1] has been isolated from licorice as a sole example of naturally occurring glucurono-glucuronide. We now report the isolation of a new glycoside from the leaves of Clerodendron trichotomum Thunb. (Verbenaceae, Japanese name is kusagi), which is acacetin-7-β-D-glucurono-β-(1→2)-D-glucuronide (I). The sugar part of this compound is the same as that of glycyrrhizin.



I R=H
II R=CH_3
III R=C_2H_5

The glycoside (I), $C_{28}H_{28}O_{17}$, m.p. 191-205°(decomp.), λ_{max}^{EtOH} 270.2 mμ (29,800), 326 (3,200), $[\alpha]_D^{22}$ -48° (1.3 % pyridine solution) is obtainable in 0.3 % yield from the air-dried leaves and gave a dimethyl ester (II), m.p. 256°(decomp.) and its hexaacetate, m.p. 234-235°, the NMR spectrum ($CDCl_3$) of which showed two ester methyl groups (3.60 and 3.63 ppm), one aromatic methoxygroup (3.82 ppm) and six acetoxy groups [1.98 (6H), 2.01 (6H), 2.08 (3H) and 2.39 ppm (3H, aromatic acetoxy)]. A diethyl ester (III), m.p. 240-242° and its hexaacetate, m.p. 222-223° were similarly formed. The mass spectrum of II-hexaacetate showed a parent peak, m/e 916, a diglucuronide fragment, 607.153 ($C_{24}H_{31}O_{18}^+$ requires 607.151) and an acacetin monoacetate fragment, 326.080 ($C_{18}H_{14}O_6^+$ requires 326.079).

When hydrolyzed with β-D-glucuronidase the glycoside gives acacetin and glucuronic acid detected by circular paper chromatography. The UV spectrum of the glycoside is unchangable on addition of sodium acetate[2] showing that

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a sugar part should be attached to 7-position of acacetin. Methylation of the glycoside followed by hydrolysis affords apigenin-4',5-dimethyl ether, m.p. 262-263°.

In order to determine the linkage between the two molecules of glucuronic acid the dimethyl ester (II) was reduced by sodium borohydride and then methylated twice by Hakomori's procedure.[3] Hydrolysis was performed by 2.5 N trifluoroacetic acid in a sealed tube and the methylated sugars obtained were detected by gas chromatography (GLC) after reduction with sodium borohydride to avoid peaks due to α and β anomers.[4]

GLC detection was achieved in three derivatives, trimethylsilyl ether (TMS), trifluoroacetate (TFA) and acetate (Ac), and two peaks corresponding to 2,3,4,6-tetra-O-methyl-D-glucitol and 3,4,6-tri-O-methyl-D-glucitol were detected in all derivatives as shown in Table I. Therefore, the structure of the glycoside (I) was assigned as acacetin-7-β-D-glucurono-β-(1→2)-D-glucuronide.

Table I. GLC Relative Retention Time

Derivatives	TMS		TFA	Ac	
Column	A	B	B	A	B
Column temperature	160°	140°	110°	180°	160°
2,3,4,6-Tetra-O-methyl-D-glucitol	1.00 (6.80 min.)	1.00 (13.30)	1.00 (13.80)	1.00 (5.30)	1.00 (7.00)
3,4,6-Tri-O-Me-D-glucitol	1.265	1.401	0.673	1.523	1.457
2,4,6-Tri-O-Me-D-glucitol	1.280	1.419	0.699	1.512	1.464
2,3,6-Tri-O-Me-D-glucitol	1.290	1.422	0.687	1.695	1.562
O-Methyl-D-glucitols obtained from I	1.00 1.266	1.00 1.404	1.00 0.673	1.00 1.522	1.00 1.455

A: 2% Silicone GE XF-1105 (2.0 m × 4 mm i.d.), B: 2% Silicone OV-1 (1.5 m × 4 mm i.d.). Carrier gas: N_2 60 ml/min. Temperature: injection port 230°, detector 230°.

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