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SYNTHESIS OF SOME FLAVONOID GLUCOSIDES OF *TRIFOLIUM SUBTERRANEUM*

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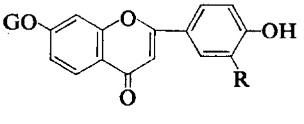
Abstract—4′,7-Dihydroxyflavone-7-glucoside (I), geraldone-7-glucoside (II), 3′,4′,7-trihydroxyflavone-7-glucoside (III), homobutein-4-glucoside (IV) and geraldol-4′-glucoside (V), which were recently reported to be present in *Trifolium subterraneum* L., have been synthesized. As the properties of the synthetic glucosides (IV) and (V) did not agree with those of the natural products, the alternative structures, namely, homobutein-4′-glucoside (IX) and geraldol-7-glucoside (X), required consideration and consequently they have also been prepared and their properties are also different from the recorded ones.

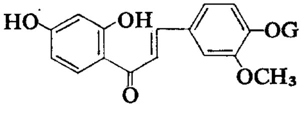
INTRODUCTION

Subterranean clover (*Trifolium subterraneum*) has long been known to contain isoflavones, coumestrol and anthocyanins but more recently, Wong and Francis[1] examined the Geraldton variety (mutant B763), which is deficient in isoflavones, and found other flavonoids, biogenetically related to the isoflavones. The chemistry of this mutant is of importance because whereas isoflavones and coumestrol are estrogenic and have a marked effect on the physiology of cattle consuming the fodder, other flavonoids are not.

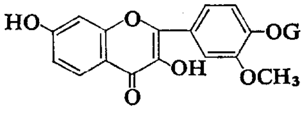
Wong and Francis[1] isolated a number of flavonoid glycosides from the leaves of the Geraldton variety of subterranean clover; nine of them (including I–V) were characterized by chromatographic and spectroscopic methods and several aglycones were synthesized. In all cases the sugar component was glucose and the general chromatographic behaviour of the glucosides suggested that they were monoglucosides. Since they were isolated in very small quantities, only spectral data were used to assign the position of the glycosidic linkage. Among these, the glucosides I and III were first reported a little earlier by Markham and Mabry[2] from *Baptisia lacontei.*



(I) R=H
(II) R=OCH_3
(III) R=OH



(IV)



G = glucosyl

(V)

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[1] E. Wong and C. M. Francis, *Phytochem.* **7**, 2123 (1968).

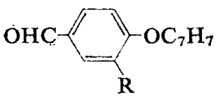
[2] K. R. Markham and T. J. Mabry, *Phytochem.* **7**, 791 (1968).

Synthesis of the five new compounds (I–V) has now been attempted, as they are closely related to the glucosides of another leguminous plant, *Butea monosperma*, some of which have been recently synthesized in this laboratory.[3]

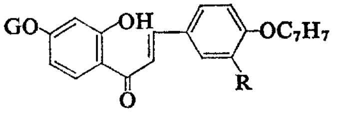
RESULTS

Several methods are available for the synthesis of flavone-7-glucosides. Direct condensation of the acetylglucosylbromide with the aglycone is known to give the 7-glucoside (e.g. luteolin-7-glucoside)[4]; however, complex mixtures are often obtained and the method is by no means unambiguous. Another method involves the oxidation of the corresponding flavanone glycoside with iodine and KOAc (e.g. diosmin),[5] but this method is convenient only when the required flavanone is readily available. Transacylation followed by glycosidation is an established method for the synthesis of flavone-7-glycosides (e.g. cosmosiin)[6] and this requires the synthesis of the aglycones to start with.

For the synthesis of the glucosides I–III, which all possess only the 7-oxygen function in the A-ring, resacetophenone-4-*O*-β-D-(tetra-*O*-acetyl)-glucoside was condensed with the suitably substituted benzaldehydes (VI) to give the chalcones (VII), which were conveniently oxidized to (VIII) with SeO_2 in good yields. This direct oxidation of chalcone glycosides to flavone glycosides with SeO_2 has not been described previously. The flavones (VIII) were debenzylated by hydrogenolysis over Pd-C, and the flavone glucosides (I–III) thus obtained, had the same R_f values, spectra and fluorescence as recorded for the natural compounds (see Table 1) but direct comparison has not been possible.

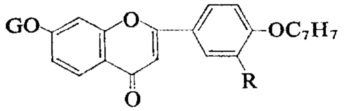


(VI)



(VII)

(a) $R=H$
(b) $R=OCH_3$
(c) $R=OC_7H_7$



(VIII)

Condensation of resacetophenone with glucovanillin gave 2′,4′-dihydroxy-3-methoxy-4-glucosidoxychalcone (IV), which when treated with alkaline H_2O_2 yielded 3′-methoxy-4′-glucosidoxy-7-hydroxyflavonol (V); u.v. spectral data of the synthetic glucosides agreed with the expected structures, but the synthetic compounds had properties widely different from those of the natural products (see Table 1). Since the structures of the aglycones of the natural products were confirmed by comparison with authentic samples, the alternative

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[3] S. R. GUPTA, B. RAVINDRANATH and T. R. SESHADRI, *Phytochem.* **9**, 2231 (1970).
[4] L. HÖRHAMMER, L. FARKAS, H. WAGNER and J. OSTERMAYER, *Acta Chim. Hung.* **40**, 463 (1964).
[5] G. ZEMPLEN and R. BOGNAR, *Ber. Deut. Chem. Ges.* **76**, 452 (1943).
[6] M. NOGRADI, L. FARKAS, H. WAGNER and L. HÖRHAMMER, *Chem. Ber.* **100**, 2783, (1967).

TABLE 1. THE PROPERTIES OF NATURAL AND SYNTHETIC COMPOUNDS

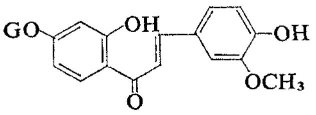
	R_f (paper) BAW (6:1:2)		R_f (paper) 30% HOAc		U.v. (EtOH)		U.v. (EtOH–NaOH)		U.v. (EtOH–AlCl$_3$)	
	Nat.	Syn.	Nat.	Syn.	Nat.	Syn.	Nat.	Syn.	Nat.	Syn.
4′,7-Dihydroxyflavone-7-glucoside (I)	0·55	0·55	—	—	329	328	390	393		
					254	255	305	305	—	—
					230	230	295	295		
4′,7-Dihydroxy-3′-methoxy-flavone-7-glucoside (II)	0·42	0·42	—	—	341	340	410	410–415		
					310*	310*	297	300	—	—
					233	233				
3′,4′,7-Trihydroxyflavone-7-glucoside (III)	0·36	0·34	0·49	0·49	343	344	403	405		
					307*	308	295	295–300	—	—
					243*	242*				
Homobutein-4-glucoside (IV)	—	—	0·43	0·63	380	369	440†	400‡	420	415
					262	257	275			263
Geraldol-4′-glucoside (V)	0·40	—	0·48	0·56	358	358	430	400	416	418
					320*	245*	278		265	320
					248					257
Homobutein-4′-glucoside (IX)	—	0·66	—	0·57		382				432
					—	310	—	475	—	272
						262				
Geraldol-7-glucoside (X)	—	0·41	—	0·47	—	362		447		425
						318	—	278	—	270
						250				

* Shoulder.

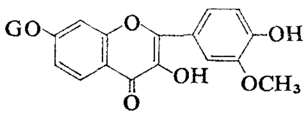
† Decrease in intensity.

‡ Increase in intensity.

structures for the glucosides, namely, homobutein-4′-glucoside (IX) and geraldol 7-glucoside (X) require consideration and therefore they have also been synthesized.



(IX)



(X)

Condensation of resacetophenone-4-*O*-glucoside tetraacetate with vanillin has yielded 2′,4-dihydroxy-3-methoxy-4′-glucosidoxychalcone (IX), but again the properties are different from those recorded for the natural chalcone glucoside. The synthetic chalcone glucoside (IX), on treatment with alkaline H_2O_2 has yielded 3′-methoxy-4′-hydroxy-7-glucosidoxyflavonol (X), whose R_f values are the same as those of the naturally occurring flavonol glucoside but the shifts in u.v. spectrum are not in agreement (see Table 1).

EXPERIMENTAL

M.ps are uncorrected. U.v. spectra are recorded in ethanol and log ϵ values are given in brackets. I.r. spectra are recorded as KBr discs; only main peaks are cited. NMR spectra are recorded in $CDCl_3$ with TMS as internal standard.

2′-*Hydroxy*-4-*benzyloxy*-4′-*glucosidoxychalcone* (*VIIa*)

Resacetophenone 4-*O*-β-D-(tetra-*O*-acetyl)glucoside (m.p. 130–2°)[7] (2 g) and *p*-benzyloxybenzaldehyde (VIa) (1·1 g) in ethanol (25 ml) was treated with aq. KOH (4 g in 4 ml water). After 36 hr at room temp. the mixture was diluted with water, washed with ether, acidified under cooling and the yellow solid, which separated was filtered. It formed yellow needles from dioxan, 1·2 g; m.p. 220–2°; λ_{max} 368 sh, 360, 250 nm; ν_{max}^{KBr} 3600–3350, 3000, 2940, 1640, 1610 sh, 1585 sh, 1540, 1510, 1408, 1380, 1355, 1325, 1300, 1280, 1250, 1220, 1170, 1135, 1115, 1080, 1050, 1015, 1000, 990, 972, 925, 872, 845, 810, 745 and 700 cm^{-1}. (Found: C, 66·1; H, 5·6. $C_{28}H_{28}O_9$ required C, 66·1; H 5·5%.)

4′-*Benzyloxy*-7-*glucosidoxyflavone* (*VIIIa*)

The above chalcone (VIIa) (0·5 g) in a mixture of dioxan (20 ml) and amyl alcohol (5 ml) was refluxed with freshly sublimed SeO_2 (1 g) for about 100 hr. It was then filtered and cooled. The pale coloured solid which separated out was filtered and recrystallized from ethanol, yielding colourless needles 0·25 g; m.p. 228–9°, sintering at 200°; λ_{max} 325 and 255 nm; ν_{max}^{KBr} 3570, 3000–2900, 1620, 1560, 1510, 1450, 1430, 1390, 1305, 1250, 1188, 1105, 1070, 1047, 1030, 916, 870, 835, 823, 730 and 690 cm^{-1}. (Found: C, 61·5; H, 5·6. $C_{28}H_{26}O_9 . 2H_2O$ required C, 62·0; H, 5·5%.)

4′-*Hydroxy*-7-*glucosidoxyflavone* (*I*)

The flavone (VIIIa) (200 mg) in a mixture of AcOH (25 ml) and methanol (25 ml) was hydrogenated over Pd-C (5%, 200 mg) for 5 hr. The solution was filtered, evaporated and the residue crystallized from aq. acetic acid, yielding colourless needles, 50 mg; m.p. 245–7° d; λ_{max} 328 (3·95), 315 (sh), 255 (3·63) and 230 (3·94) nm; ν_{max}^{KBr} 3460, 2985, 1650, 1635, 1575, 1515, 1450, 1380, 1250, 1212, 1190, 1175, 1143, 1105, 1080, 1045, 1010, 990, 910, 835 and 825 cm^{-1}. (Found: C, 60·1; H, 5·0. $C_{21}H_{20}O_9$ required C, 60·5; H, 4·8%.)

2′-*Hydroxy*-3-*methoxy*-4-*benzyloxy*-4′-*glucosidoxychalcone* (*VIIb*)

Resacetophenone-4-glucoside tetraacetate (2 g) was condensed with benzylvanillin (VIb) under the conditions employed for (VIIa); the yellow crystalline solid (VIIb) thus obtained was recrystallized from ethanol, yielding deep yellow needles, 1·8 g; m.p. 173–5°; λ_{max} 377 and 260 nm; ν_{max}^{KBr} 3580, 2940, 1640, 1580, 1510, 1450, 1410, 1370, 1350, 1265, 1208, 1175, 1135, 1078, 1025, 970, 850, 795, 755, 740 and 700 cm^{-1}. (Found: C, 65·1; H, 5·5. $C_{29}H_{30}O_{10}$ required C, 64·7; H, 5·6%.)

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[7] L. Reichel and J. Steudel, *Liebigs Ann.* **553,** 83 (1942).

3′-Methoxy-4′-benzyloxy-7-glucosidoxyflavone (VIIIb)

The chalcone (VIIb) (1 g) was refluxed for 14 hr in amyl alcohol (15 ml) with SeO_2 (1·2 g). The hot amyl alcohol solution on filtering and cooling deposited a yellow solid, which was chromatographed on silica gel yielding colourless needles, 350 mg; m.p. 210–1° (EtOH); λ_{max} 335 and 235 (sh) nm; v_{max}^{KBr} 3580, 2940, 1634, 1520, 1450, 1425, 1390, 1275, 1212, 1180, 1100, 1070, 1015, 862, 825, 810, 732 and 690 cm^{-1}. (Found: C, 63·0; H, 5·9. $C_{29}H_{28}O_{10}$ required C, 62·8; H, 5·5%.)

3′-Methoxy-4′-hydroxy-7-glucosidoxyflavone (II)

The flavone (VIIIb) (250 mg) was hydrogenated in MeOH + AcOH, over Pd-C (5%, 200 mg) for 12 hr· The product was a 1 : 1 mixture of the starting material and the debenzylated compound. They were separated by column chromatography over silica gel and 3′-methoxy-4′,7-dihydroxy flavone-7-glucoside (II) was obtained as pale yellow needles, 25 mg; m.p. 188–90° (EtOH); λ_{max} 340 (4·27), 310 (sh) and 233 (4·26) nm; v_{max}^{KBr} 3510, 2985, 1630, 1515, 1450–1430, 1370, 1300, 1265, 1175, 1143, 1130, 1100–1075, 1047, 1031, 990, 930, 900, 855, 815, 757 and 685 cm^{-1}. (Found: C, 56·4; H, 5·5. $C_{22}H_{20}O_{10}.H_2O$ required C, 56·8; H, 5·2%.)

2′-Hydroxy-3,4-dibenzyloxy-4′-glucosidoxychalcone (VIIc)

Resacetophenone-4-glucoside tetraacetate (2 g) was condensed with 3,4-dibenzyloxybenzaldehyde (VIc) in aq. ethanolic KOH (10%, 40 ml) and worked up as usual, yielding yellow needles from ethanol, 1·8 g; m.p. 171–3°; λ_{max} 376, 260 and 245 nm; v_{max}^{KBr} 3333, 1640, 1575, 1515, 1470, 1420, 1370, 1265, 1212, 1135, 1070, 1015, 980, 850, 805, 735 and 700 cm^{-1}. (Found: C, 67·5; H, 5·8. $C_{35}H_{34}O_{10}.\frac{1}{2}H_2O$ required C, 67·6; H, 5·7%.)

3′,4′-Dibenzyloxy-7-glucosidoxyflavone (VIIIc)

The above chalcone (VIIc) (1·5 g) was refluxed with SeO_2 (2 g) in amyl alcohol (25 ml) for 19 hr, filtered and cooled. The pale yellow solid which separated was filtered and recrystallized from ethanol, yielding pale coloured microcrystalline solid, 750 mg; m.p. 212–3°; λ_{max} 336, 320 (inflex) and 235 (sh) nm; v_{max}^{KBr} 3333, 2865, 1626, 1600, 1515, 1450, 1380, 1335, 1265, 1175, 1143, 1105 (sh), 1075, 1020, 850, 815, 740 and 700 cm^{-1}. (Found: C, 66·4; H, 5·8. $C_{35}H_{32}O_{10}.H_2O$ required C, 66·6; H, 5·4%.)

3′,4′-Dihydroxy-7-glucosidoxyflavone (III)

The benzyl ether (VIIIc) (400 mg) was hydrogenated in MeOH + HOAc over Pd-C (10%, 250 mg), filtered, evaporated and the residue crystallized from aq. ethanol, yielding (III) as pale yellow shining needles, 125 mg; m.p. 190–1°; $FeCl_3$-green; λ_{max} 344 (3·94), 308 (3·81), 242 (sh) and 227 (sh) nm; v_{max}^{KBr} 3570, 2985, 1626, 1550, 1513, 1450, 1390, 1307, 1270, 1238, 1190, 1140–1030, 1080 (broad), 990 and 820 cm^{-1}. (Found: C, 56·4; H, 4·9. $C_{21}H_{20}O_{10}.H_2O$ required C, 56·0; H, 4·9%.) Acetate: colourless needles from ethanol m.p. 147–8°. NMR spectrum: δ 2·07 (12H, four acetoxyls of glucose), δ 2·33 (3H) and δ 2·36 (3H) (for 3′ and 4′-acetoxyls), δ 6·73 (1H, C_3-H), δ 7·17 (broad, 2H, C_6 and C_8-H), δ 8·74 (1H, d, $J = 9$ c/s, $C_{5'}$-H), δ 7·75 (2H, m, $C_{2'}$ and $C_{6'}$-H) and δ 8·2 (1H, d, $J = 9$ c/s, C_5-H).

2′,4′-Dihydroxy-3-methoxy-4-glucosidoxychalcone (IV)

A mixture of resacetophenone (1 g) and vanillin-*O*-β-D-glucoside (m.p. 188–90°) (2 g) was suspended in ethanol (10 ml) and aq. KOH (20 g in 20 ml water) was added with cooling and stirring. After 72 hr at room temp., the mixture was diluted with ice–water, acidified with HCl under vigorous cooling and extracted with ether and EtOAc. The EtOAc extract on concentration and crystallization from aq. methanol yielded homobutein-4-glucoside (IV) as deep yellow needles; 100 mg. m.p. 178–80°; λ_{max} 369 (4·24) and 257 (3·88) nm; v_{max}^{KBr} 3570, 3010, 1640, 1595, 1560, 1515, 1450, 1355, 1265, 1205, 1134, 1075, 1025, 975, 850 and 805 cm^{-1} (Found: C, 54·8; H, 5·8. $C_{22}H_{24}O_{10}.2H_2O$ required C, 54·5; H, 5·8%.)

3′-Methoxy-4′-glucosidoxy-7-hydroxyflavonol (V)

Homobutein-4-glucoside (IV, 50 mg) was dissolved in methanol (1 ml) and aq. NaOH (20%; 2 ml) was added. The red solution was warmed to about 60° and 30% H_2O_2 was added drop by drop till the colour of the solution changed to deep yellow. After 5 min, the mixture was diluted with water, acidified and cooled. The yellow solid which separated was filtered and recrystallized from MeOH–EtOAc, yielding pale yellow needles, 20 mg; m.p. 232–4° (sintering at 225°); $FeCl_3$—green; λ_{max} 358 (4·40), 320 (sh), 245 (sh) and 225 (sh) nm; +NaOAc, 358, 320 (sh), 260 (sh), 245 (sh), and 232 nm; v_{max}^{KBr} 3333, 2900, 1615, 1575 (sh), 1515, 1470, 1430, 1340, 1265, 1250, 1212, 1198, 1150, 1135, 1105, 1070, 1020, 905, 850, 825, 810, 775 and 705 cm^{-1}. (Found: C, 55·3; H, 5·4.$C_{22}H_{22}O_{11}.H_2O$ required C, 55·0; H, 5·0%).

2′,4-Dihydroxy-3-methoxy-4′-glucosidoxychalcone (IX)

To resacetophenone-4-glucoside tetraacetate (3·6 g), suspended in ethanol (10 ml) was added aq. KOH (60%, 25 ml), followed by vanillin (1·4 g) and again aq. KOH (10 ml). The mixture was made homogenous by adding a few drops of water and ethanol. After 3 days at room temperature, the mixture was acidified with

HCl under cooling and filtered. Homobutein-4′-glucoside (IX), thus obtained (1 g) crystallized from aq. ethanol yielding deep yellow needles, m.p. 163–5°; λ_{max} 382 (3·92), 310 (3·63) and 262 (3·82) nm; υ_{max}^{KBr} 3390, 2940, 1650, 1575, 1515, 1429, 1370, 1280, 1250, 1220, 1130, 1075, 1030, 1020 (sh), 980, 970, 900, 850 and 805 cm^{-1}. (Found: C, 58·4; H, 5·6. $C_{22}H_{24}O_{10}$ required: C, 58·9; H, 5·4%.)

3′-Methoxy-4′-hydroxy-7-glucosidoxyflavonol (X)

Homobutein-4′-glucoside (IX) (100 mg) was dissolved in hot methanol, aq. NaOH (20%, 2 ml) added and the mixture treated at 60° with H_2O_2 (30%) till the red solution changed to deep yellow. After 5 min the solution was diluted with water, acidified and cooled, when the flavonolglucoside (X) crystallized as pale yellow needles, 40 mg; m.p. 235–7° (loss of water at 145°); λ_{max} 362 (3·98), 318 (3·82) and 250 (3·95) nm; υ_{max}^{KBr} 3390, 2940, 1615, 1563, 1515, 1460, 1440, 1408, 1370, 1325, 1280, 1260, 1205, 1190, 1135, 1105–1010, 1075, 855, 830, 805, 780, 705 and 685 cm^{-1}. (Found: C, 52·7; H, 5·1. $C_{22}H_{22}O_{11}.2H_2O$ required C, 53·0; H, 5·2%.)