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CUCURBITACEAE

CHEMICAL EXAMINATION OF *LUFFA ECHINATA*

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Abstract—In the fruit of *Luffa echinata*, the rare flavonoid, chrysoeriol occurs along with its 7-glucoside and 7-apioglucoside. Two other flavones, apigenin and luteolin are also found in minor amounts as glycosides. As already reported, elaterin and isocucurbitacin-B occur along with the bitter elaterin-2-glucoside and β-sitosterol glucoside. Apiose in combined form is now first reported in Cucurbitaceae.

Luffa echinata, is a common Indian herbal drug. Earlier chemical studies[1-3] on the intensely bitter fruits have shown the presence of elaterin, isocucurbitacin-B, gypsogenin, oleanolic acid and glucose, xylose and rhamnose. Our present study has revealed the marked presence of flavonoids.

Using air dried fruits, successive extracts with light petroleum, ether, acetone, alcohol and alcohol–water mixture (60:40) were examined. The light petroleum extract, on column chromatography, yielded elaterin and isocucurbitacin-B. The ether extract contained these two compounds along with a bitter glycoside giving positive Molisch's test and L-B colour reaction. Hydrolysis of this glycoside was effected with the enzyme of the elaterase type obtained from the plant material yielding elaterin and glucose. Quantitative acid hydrolysis and permethylation followed by hydrolysis proved that the compound was elaterin 2-*O*-β-D-glucopyranoside.

The acetone extract on column chromatography gave mainly this elaterin glucoside. However, the earlier fractions contained a mixture of it and a flavone glycoside which only after acetylation, could be separated by preparative TLC. The flavone glycoside acetate (small yield) on acid hydrolysis yielded chrysoeriol and glucose (1:1) and its identity as chrysoeriol 7-monoglucoside acetate was confirmed by direct comparison. The natural occurrence of this 7-glucoside in species of *Pyrus* has recently been noted by chromatographic and spectroscopic methods.[4]

From the alcohol concentrate by the addition of ether another yellow flavone glycoside separated. It gave, on acid hydrolysis, apiose and glucose[5] and chrysoeriol.[6] The u.v. data of the glycoside are as follows: λ_{max}^{EtOH} 253, 270, 290 (inf.), 349 nm; +NaOAc no shift; +NaOAc + H_3BO_3 no shift; +$AlCl_3$ 254, 279, 295 (inf.), 355, 390 nm; +NaOEt 270, 310 (inf.) 402 nm. Absence of shift with NaOAc showed that the sugar was in the 7-position. Complete methylation of the glycoside followed by hydrolysis as well as permethylation followed by hydrolysis confirmed that the compound was chrysoeriol 7-*O*-β-D-glucopyranosidyl (2 → 1) D-apiofuranoside. The NMR spectra of the acetate of the aglycone and the

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glycoside (Table 1) lend further support. Final confirmation was provided by direct comparison with graveobioside-B isolated from celery seeds.

TABLE 1. NMR SPECTRA OF ISOLATED COMPOUNDS

Compound	δ Values	No. of protons	Multiplicity	Assignments
	2·32	6	S	2-$OCOCH_3$
	2·45	3	S	1-$OCOCH_3$
	3·91	3	S	1-OCH_3
Chrysoeriol acetate	6·60	1	D	C_6-H
	6·85	1	S	C_3-H
	6·87	1	D	C_8-H
	7·15	1	D	$C_{5'}$-H
	7·35	2	M	$C_{2'}$ & $C_{6'}$-H
	1·99	3	S	1-$OCOCH_3$
	2·02	6	S	2-$OCOCH_3$
	2·04	3	S	1-$OCOCH_3$
	2·13	3	S	1-$OCOCH_3$
Graveobioside-	2·15	3	S	1-$OCOCH_3$
B acetate*	2·36	3	S	1-$OCOCH_3$
	2·44	3	S	1-$OCOCH_3$
	3·96	3	S	1-OCH_3

* The signals for the aromatic protons were almost same as for the aglycone acetate.

The ether solution (M) on column chromatography gave chrysoeriol and β-sitosterol glucoside. The aq. alcohol extract yielded a mixture of flavonoid glycosides containing mainly graveobioside-B. Its hydrolysis gave chrysoeriol as the major and apigenin and luteolin as the minor aglycones.

EXPERIMENTAL

All identities were confirmed by direct comparison using colour reactions, mixed m.ps and superimposable i.r. spectra. M.ps are uncorrected and were determined both on a Koffler-block and in H_2SO_4 bath. Paper chromatography was carried out on Whatman No. 1 filter paper, TLC and column chromatography using NCL grade silica gel.

Light petroleum extract: (Elaterin and isocucurbitacin-B). The powdered air dried fruits (1 kg) were soxhleted with light petroleum (b.p. 60°–80°) for 24 hr. The crude oily concentrate on column chromatography gave in 2% MeOH in $CHCl_3$ eluate a colourless solid (200 mg), needles from MeOH, m.p. 234°; $[\alpha]_D^{25}$–57° ($CHCl_3$); λ_{max}^{EtOH} 234 and 267 (shoulder) nm. Its diacetate, needles from Et_2O had m.p. 119°. Its identity as elaterin was confirmed by a direct comparison. Later fractions yielded another colourless substance (50 mg); no Fe^{3+} reaction; m.p. 221–2° (d) after crystallization from methanol; $[\alpha]_D^{25}$ + 44° ($CHCl_3$), λ_{max}^{EtOH} 229 nm; identified as isocucurbitacin-B[7].

Ether extract: (Elaterin, isocucurbitacin-B, and elaterin glucoside). The Et_2O extract on column chromatography yielded the above two free cucurbitacins from MeOH–$CHCl_3$ (2:98) eluate. A glycoside was obtained in MeOH–$CHCl_3$ (10:90) eluate which was crystallized from ethyl acetate–Et_2O mixture (500 mg). It gave no Fe^{3+} reaction. It was hydrolysed by the enzyme isolated from the same plant material as follows: Crushed fruits (15 g) were soaked in H_2O (25 ml) overnight. The extract was filtered and cold EtOH (75 ml) added. The precipitated enzyme was filtered, washed with EtOH and dissolved in H_2O (5 ml). To the glycoside (20 mg) was added the enzyme solution (0·5 ml) and the mixture was kept for 48 hr at 30°. The product was extracted with Et_2O and the extract washed with H_2O, dried (Na_2SO_4) and concentrated. It gave strong Fe^{3+} colour and showed a spot corresponding to elaterin on TLC. The sugar was identified as glucose. (Found:

[7] S. M. KUPCHAN, H. A. GRAY and D. M. GROVE, *J. Med. Chem.* **10**, 337 (1967).

glucose, 22·5; $C_{38}H_{54}O_{13}$ requires for 1 mole glucose 25·0%.) Permethylation using Hakomori's[8] procedure followed by hydrolysis gave 2,3,4,6-tetra-*O*-methyl glucose. These results agreed with its being elaterin 2-*O*-β-D-gluco-pyranoside.[9]

Acetone extract: (*Elaterin glucoside, chrysoeriol 7-glucoside and its apioglucoside*). The acetone concentrate was column chromatographed. MeOH–$CHCl_3$ (10:90) eluate yielded a mixture showing two spots on TLC of which the major was elaterin glucoside while the other gave flavonoid reactions. The mixture was acetylated and the acetates were separated by preparative TLC; flavonoid acetate (30 mg) had m.p. 212°. (Found: C, 56·9; H, 5·0. $C_{34}H_{34}O_{17}$ requires C, 57·1; H, 4·8%.) Hydrolysis of it (20·02 mg) with boiling 7% aq. H_2SO_4 for 4 hr gave chrysoeriol (8·22 mg), m.p. > 310°. Glucose was identified by paper chromatography. (Found: chrysoeriol 41·1; $C_{34}H_{34}O_{17}$, should yield the aglycone 42·0%.) This flavonoid acetate agreed with the acetate of the 7-glucoside of chrysoeriol obtained by partial hydrolysis of graveobioside-B. A minor glycoside identified as graveobioside-B was isolated from 25% MeOH in $CHCl_3$ eluate.

Alcohol extract: (*Graveobioside-B, chrysoeriol and β-sitosterol glucoside*). The concentrate was dissolved in methanol and the insoluble inorganic matter removed and excess Et_2O added. The precipitated solid was filtered. The ether soluble (M) is discussed later. The yellow solid was redissolved in MeOH. On standing for a few hours, the separated gelatinous product was filtered and several crystallizations from MeOH afforded pale yellow needles of graveobioside-B melting at 211–12° (1 g), $[\alpha]_D^{25}$ — 104·43 (pyr.), acetate m.p. 247°.

Hydrolysis of the glycoside (500 mg) with boiling 7% MeOH–H_2SO_4 (15 ml) for 4 hr gave chrysoeriol, needles from EtOH (200 mg). The sugar moieties were found to be glucose and apiose by comparison with authentic samples prepared by the hydrolysis of lanceolarin.[5] Partial hydrolysis[10] yielded chrysoeriol 7-monoglucoside as yellow crystals m.p. 238°; $[\alpha]_D^{25}$ — 44·16° (pyr.). Acetate m.p. 212°. The aq. filtrate contained apiose. Further hydrolysis with 7% MeOH–H_2SO_4 for 4 hr gave chrysoeriol and glucose. Enzymatic hydrolysis of this partial glycoside with almond emulsin also gave the same products.

Complete methylation of the glycoside with excess of Me_2SO_4 and K_2CO_3 for 80 hr gave a brown oily product which was chromatographed when the pure methylated glycoside (m.p. 286–8°) was obtained. In earlier work this stage was not isolated and characterized. It gave no Fe^{3+} reaction. (Found: C, 55·6; H, 5·5. $C_{29}H_{34}O_{15}$ requires C, 55·9; and H, 5·3%.) Its hydrolysis with 7% aq. H_2SO_4 for 3 hr gave 5,3′,4′ trimethyl luteolin,[10] m.p. 283–4°.

Permethylation[8] of the glycoside (15 mg) with NaH, DMSO and CH_3I was carried out. The product, obtained as a syrup was hydrolysed using Kiliani[11] mixture. The methylated sugars were identified as 3,4,6-trimethyl glucose and 2,3,4-trimethyl apiose by paper chromatography. This method is essentially simple and it has been earlier employed for saponins. In order to check its applicability to flavonoids, a trial was made with rutin when satisfactory results were obtained.

The ether soluble portion (M) on column chromatography gave a yellow compound from MeOH–$CHCl_3$ (2:98) eluate, identified as chrysoeriol. The other compound isolated from 5% MeOH in $CHCl_3$ eluate was a white crystalline solid (100 mg) m.p. 291°, giving positive Molisch's test. Hydrolysis with aqueous acid gave β-sitosterol, m.p. 134° and glucose; the glucoside itself agreed with authentic β-sitosterol glucoside.

Aq. alcohol-extract. The extract (6 l.) when concentrated under reduced pressure to about 1 l. yielded a solid on cooling (7 g). Its hydrolysis with 7% MeOH–H_2SO_4 for 4 hr yielded the aglycone mixture containing mainly chrysoeriol which was removed by crystallization. The mother liquor showed three spots corresponding to chrysoeriol, apigenin and luteolin. Apigenin could be separated by preparative TLC (15 mg), m.p. > 310°, λ_{max}^{EtOH} 269, 336 nm. Its triacetate, needles from ethyl acetate–petroleum ether had m.p. 180°. The presence of luteolin could be detected only by a TLC comparison of the flavone mixture as well as of the acetates with authentic luteolin and its acetate.

Isolation of graveobioside-B from celery seeds: The procedure described below is more convenient than the earlier one[10] for the isolation of graveobioside-B. Powdered celery seeds (200 g) were extracted with boiling 95% alcohol and the concentrate dissolved in MeOH. Precipitations with excess Et_2O removed waxy materials, giving a yellow powder. This was column chromatographed; earlier fractions of MeOH–$CHCl_3$ (8:92) contained graveobioside-B in major amounts. These were combined and rechromatographed when an almost pure sample was obtained which was recrystallized from alcohol (m.p. 214–16°).

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