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Phytochemistry, 1979, Vol. 18, pp. 365–366. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0365 $02.00/0

A NEW ISOFLAVONE GLUCOSIDE FROM *CAJANUS CAJAN*

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(*Received* 10 *July* 1978)

Key Word Index—*Cajanus cajan*; Leguminosae; 5,7,2′-trihydroxyisoflavone 7-glucoside; genistein; sitosterol glucoside; sitosterol; lupeol; α-amyrin; β-amyrin.

Previous investigations [1, 2] of the genus *Cajanus* have established the structures of some new prenylated flavonoids. We now report the isolation and identification of a new isoflavone glucoside, together with 5,7,4′-trihydroxyisoflavone (genistein), and the triterpenoids sitosterol and its glucoside, lupeol, and α- and β-amyrin.

The Me_2CO-insoluble portion of the alcoholic extract of the root bark on column chromatography over Si gel gave two fractions. PC (Whatman 3 mm) of the first fraction using 10% aqueous HOAc yielded the new glucoside as colourless needles (from dilute alcohol) mp 200–203° (d); PC, R_f: 0.89 (BAW, 4:1:5); 0.42 (10% aq. HOAc); TLC, R_f: 0.51 (Si gel, EtOAc–MeOH–H_2O, 100:16.5:13.5). It gave an olive green colour with alcoholic $FeCl_3$. IR(KBr) showed strong absorptions at 1666 cm^{-1} (chelated >C=O) and 3573 cm^{-1}. MS exhibited M^+ 432.1056 ($C_{21}H_{20}O_{10}$) and an aglycone peak at *m/e* 270 (100%) formed by the elimination of a six carbon sugar. These observations coupled with the UV spectrum (λ_{max}^{MeOH} 260, 320; +$AlCl_3$, 240, 270, 365; +$AlCl_3$–HCl 240, 270–75, 365; +NaOAc, 260 nm) indicated it to be an isoflavone glycoside. Acid hydrolysis gave an aglycone which crystallized from MeOH as long straw yellow coloured needles mp 222–24° sintering at 190°; TLC, R_f: 0.52 (Si gel, C_6H_5Me–HCO_2Et–HCO_2H, 5:4:1); 0.78 (Si gel, EtOAc–C_6H_6, 3:7); M^+ 270.0524, $C_{15}H_{10}O_5$; and a sugar unit, identified as glucose by PC. In the MS of the aglucone, the retro–Diels-Alder cleavage produces two ketene fragments at *m/e* 153 (88) and 152 (80), and one acetylene fragment at *m/e* 118 (78) suggesting it to be an isoflavone with two hydroxyls in ring A and one hydroxyl in ring B [3]. The fragment at *m/e* 253 (23), indicated the presence of the hydroxyl in ring B at 2′ position. The driving force for the elimination of the 2′ hydroxyl to give the ion at *m/e* 253 (M-17) could be provided by the resonance stabilization of the resulting furan structure. UV spectrum [λ_{max}^{MeOH} (log ε): 260 (4.65), 325 (3.81); +$AlCl_3$, 270, 370; +$AlCl_3$–HCl, 270–75; 375; +NaOAc, 270–75, 375 nm] of the aglucone suggested the two hydroxyls in ring A to be at 5 and 7 positions. The third hydroxyl in ring B could only be at position 2′ and thus the structure 5,7,2′-trihydroxyisoflavone (4) has been assigned to the aglucone. A comparison of the UV spectra of the isomeric 5,7,3′- and 5,7,4′-trihydroxyflavones [4, 5] also favours the proposed structure. This is the first report of its natural

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occurrence and it is significant because the oestrogenic activity of a synthetic sample was found to be one fourth of that of the isomeric 5,7,4′-trihydroxyisoflavone [4].

A comparison of the UV values of the aglucone with those of the glucoside suggested the presence of the sugar moiety at position 7. Methylation of the glucoside with CH_2N_2 for 30 hr and subsequent hydrolysis gave a compound, the UV (λ_{max}^{MeOH}: 255, 315, 330, 370 nm) and the spectral shifts (NaOAc, 260, 355, 365, 375 nm) of which indicated the presence of a free 7-hydroxyl. The glucoside could also be hydrolysed with emulsin indicating the presence of a β-linkage. Therefore the new isoflavone must be 5,7,2′-trihydroxyisoflavone 7-*O*-β-D-glucoside.

The second fraction from the column on repeated crystallization from MeOH–$CHCl_3$ yielded a terpene glycoside, mp 300°(d) which was identified as sitosterol β-D-glucoside by direct comparison with an authentic sample. Column chromatography of the C_6H_6-insoluble portion of the Me_2CO extract of the root bark yielded a major flavonoid, mp 290°. The IR, UV of the compound and PMR of its acetate [6] showed it to be 5,7,4′-trihydroxyisoflavone (genistein). The C_6H_6-soluble portion of the alcoholic extract was chromatographed on Si gel. Using petrol with increasing amounts of C_6H_6 as eluent sitosterol, lupeol, and α- and β-amyrin were isolated and identified by direct comparison with authentic samples.

Acknowledgement—The authors are grateful to Prof. R. H. Thomson, University of Aberdeen, Scotland for accurate measurement of MS and molecular formulae for the new glucoside and its aglucone.

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Phytochemistry, 1979, Vol. 18, pp. 366–368. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0366 $02.00/0

PETALOSTETIN, A NEW ISOFLAVONE FROM *PETALOSTEMON CANDIDUM*

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(*Received* 10 *July* 1978)

Key Word Index—*Petalostemon candidum*; Leguminosae; petalostetin; 6,7,8-trimethoxy-3′,4′-methylenedioxyisoflavone.

The genus *Petalostemon* has received relatively little attention. *P. gattingeri* has been reported to contain derivatives of 2-(4-hydroxybenzyl)-malic acid [1] and *P. villosum* contains unspecified flavonoid(s) [2]. *Petalostemon* belongs to the Leguminosae, in which isoflavones are common [3]. It was not surprising then, that the ethanol extract of *Petalostemon candidum* provided what we have called petalostetin, $C_{19}H_{16}O_7$, which appeared to be isoflavonoid in nature. Specifically, the IR (1635, 1600 and 1520 cm^{-1}), UV(λ_{max} 264 and 320 nm) and PMR (H-2 at 7.97 ppm; see Table 1) spectra of petalostetin (**1**) immediately suggested [4] that this compound was an isoflavone. Furthermore, the PMR spectrum (Table 1, compound **1**) showed the presence of one methylenedioxy and three methoxy groups. Preparation of dihydropetalostetin (**3**) and examination of its physical data allowed all of the methoxy groups to be placed on ring A and the methylenedioxy group to be located on ring C. That is, the MS of dihydropetalostetin exhibited as its major fragmentation pathway the retro-Diels–Alder process which is typical of such isoflavanones [5].

Table 1 shows a comparison of the PMR spectra of the closely-related isoflavonoids **1**–**7**. Comparison of the spectra of the known **2**, **5** and **7** with that of petalostetin (**1**) and the spectra of the known **4** and **6** with that of dihydropetalostetin (**3**) allowed all substituents to be located precisely. Specifically, the downfield position of one of the aromatic protons of petalostetin (7.42 ppm) and of dihydropetalostetin (7.15 ppm) indicates that this proton must be at C-5 (i.e. adjacent to the carbonyl group).

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