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SHORT COMMUNICATION

STRUCTURE OF A SAFFLOWER STEROID CELLOBIOSIDE

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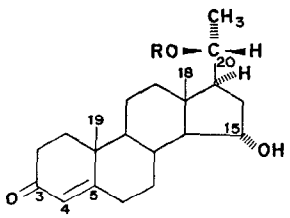
Key Word Index—*Carthamus tinctorius*, Compositae; steroid; 15α-20β-dihydroxy-Δ⁴-pregnen-3-one 20-cellobioside.

Abstract—Structural identification of a steroid diglucoside from *Carthamus tinctorius* whose aglycone is 15α,20β-dihydroxy-Δ⁴-pregnen-3-one has been completed. We have analyzed the sugar moiety of the glycoside and found it to be cellobiose, β-linked to C-20 of the aglycone.

WE HAVE previously reported the isolation of a bitter steroid glycoside occurring in safflower meal (*Carthamus tinctorius* L. Compositae). Structural analysis showed the aglycone was 15α,20β-dihydroxy-Δ⁴-pregnen-3-one (I).[1] The work now presented clearly demonstrates that the steroid occurs as the C-20β-cellobioside (II).

TLC and paper chromatographic analyses of an acid hydrolysate of the steroid glycoside showed the only sugar present was glucose. NMR analysis of the parent glycoside in DMF-d_7 at 80° showed an apparent triplet arising from a fortuitous overlap of two anomeric



(I) R=H
(II) R=β-Cellobiose
-(4-O-β-D-Glucopyranosyl-
D-Glucose)

proton doublets at δ = 4·43 (1 H) and δ = 4·36 (1 H) with couplings of 6·5 and 7·5 Hz. These couplings are characteristic of a *trans* diaxial configuration such as exists for the anomeric proton of β-glucose. In the same solvent, cellobiose also yielded an apparent triplet arising from overlapping anomeric doublets centered at δ = 4·37, also with couplings of 6·5 and 7·5 Hz. It was concluded that there may be 2 glucose units attached to the steroid aglycone and each glucose linkage is β-orientated. Further evidence came from the mass spectrum of the acetylated derivative of II which gave a molecular ion at *m/e* 992 corresponding to the calculated molecular weight of the fully acetylated steroid containing two acetylated glucose units.

Evidence that the two glucose units were mutually linked came from a partial acid hydrolysis of II. One of the five components present in the hydrolysis mixture was a reducing

[1] R. PALTER, R. E. LUNDIN and G. FULLER, *Phytochem.* **11**, 819 (1972).

disaccharide, the trimethylsilyl derivative of which was subjected to mass spectral analysis. The abundances of the mass spectral fragment peaks allows the various disaccharides to be differentiated.[2,3] The mass spectrum differed greatly from the reported spectra of laminaribose (3-*O*-β-D-glucopyranosyl-D-glucose) and gentibiose (6-*O*-β-D-glucopyranosyl-D-glucose) TMS derivatives, but was qualitatively similar to the reported spectra of both the cellobiose (4-*O*-β-D-glucopyranosyl-D-glucose) and sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) TMS derivatives. The MS of the TMS derivatives of authentic samples of each of the known disaccharides were recorded under the same conditions as the isolated disaccharide-TMS derivative. The MS peaks obtained for the isolated dissaccharide and the authentic cellobiose TMS derivatives are equal within experimental error for all masses reported to be diagnostic for the inter-monosaccharidic bond. Significant differences were found, however, in the abundances at *m/e* 918, 903, 569 and 450 between the isolated disaccharide and the authentic sophorose TMS derivatives.

Oxidation of II and subsequent acid hydrolysis gave a mono-hydroxy, mono-keto derivative which showed a marked difference in R_f from an authentic sample of 15α-hydroxyprogesterone and indicated the compound produced was 15-keto 20β-hydroxy-Δ^4-pregnen-3-one. This conclusion was confirmed by NMR analysis in $CDCl_3$. The absence of an acetyl proton peak in the vicinity of $\delta = 2{\cdot}2$ eliminated the possibility of a C-20 keto group while a three proton doublet ($J = 6$ Hz) centered at $\delta = 1{\cdot}21$ could be assigned to the C-21 methyl group with the hydroxyl on C-20 by analogy with the steroid aglycone.[1] The C-18 methyl peak occurred at $\delta = 0{\cdot}89$ and the C-19 methyl peak occurred at $\delta = 1{\cdot}21$, in agreement to within 0·005 ppm of the values calculated for 15-keto 20β-hydroxy-Δ^4-pregnen-3-one by use of the shift values of Bhacca and Williams.[4] The alternate configuration requires the same position for the C-19 resonance but the C-18 peak occurs at $\delta = 0{\cdot}70$.[4] The spectrum of an authentic sample of 15α-hydroxy-progesterone differs, as expected, from that of the steroid glucoside derivative. Thus, the structure of the isolated safflower steroid is 15α-hydroxy-Δ^4-pregnen-3-one-20β-cellobioside.

EXPERIMENTAL

NMR spectra were obtained with an internally locked Varian HR 100 Spectrometer using TMS as the internal standard. MS analyses were performed on a Consolidated Electrodynamics 21-110A Mass Spectrometer.

Complete glycoside hydrolysis. The glycoside was hydrolyzed with 0·5 N HCl for 4 hr at 100° in an evacuated sealed tube. The aglycone was removed with $CHCl_3$ and the aqueous solution deionized and examined by TLC on SiO_2 plates developed with PrOH–EtOAc–H_2O (7:2:1) and visualized with anisaldehyde spray.

Isolation of cellobiose. The glycoside was heated overnight at 100° with 0·05 N HCl and worked up as above. The neutralized aqueous portion was separated on Whatman 3 MM paper with BuOH–pyridine–H_2O (6:4:3), and cellobiose eluted from the paper with H_2O.

Oxidation and hydrolysis of the steroid glycoside. The steroid glycoside in acetone was oxidized at 15° with Jones Reagent[5] for 3 hr. The product was hydrolyzed and the ketone extracted with $CHCl_3$ and examined on TLC (SiO_2) with $CHCl_3$–acetone (9:1). The pertinent band was located under UV light, extracted with $CHCl_3$ and dried. The measured mass of the molecular ion of 15-keto 20β-hydroxy-Δ^4-pregnen-3-one was 330·2235; calculated mass for $C_{21}H_{30}O_3$ is 330·2194.

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