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A CATHARTIC LIGNAN GLYCOSIDE ISOLATED FROM *CARTHAMUS TINCTORUS*

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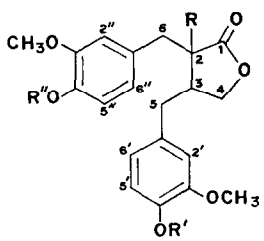
INTRODUCTION

ONE OF the many factors that prevent high protein meal from safflower (*Carthamus tinctorus* L. Compositae) from being fully utilized as a feed or food product is its cathartic effect. We found that the bitter taste of the meal was partially due to the 2,3-dibenzylbutyrolactone lignan, matairesinol monoglucoside (I).[1] We now find there is another lignan in the meal that is tasteless but which exhibits cathartic activity when tested on experimental rats. Spectroscopic analyses of this compound and its derivatives show it is an analogue of arctiin (II) having a hydroxyl group on the 2 position of the lactone and is[2] 2-hydroxy-2-(4″-β-D-glucosyl-3″-methoxybenzyl)-3-(3′,4′-dimethoxybenzyl) butyrolactone (III) or 2-hydroxyarctiin.



(I) R = H, R′ = glucosyl, R″ = H
(II) R = H, R′ = CH_3, R″ = glucosyl
(III) R = OH, R′ = CH_3, R″ = glucosyl
(IV) R = R′ = R″ = H
(V) R = R″ = H, R′ = CH_3

Recently, 2-hydroxyarctiin was reported to occur in *Trachelospermum asiaticum* var *intermedium*[3] and given the trivial name tracheloside in the belief it was identical to a compound obtained by Takano *et al.*[4–6] The Takano compound however has a molecular formula $C_{36}H_{50}O_{18}$[7] whereas 2-hydroxyarctiin is $C_{27}H_{34}O_{12}$.

[1] R. PALTER and R. E. LUNDIN, *Phytochem.* **9**, 2407 (1970).
[2] R. D. HAWORTH, *Nature, Lond.* **147**, 225 (1941).
[3] I. INAGAKI, S. HISADA and S. NISHIBE, *Phytochem.* **10**, 211 (1971).
[4] M. MIYASAKI, H. WATANABE and T. TAKANO, *J. Pharm. Soc. Japan* **78**, 879 (1958).
[5] H. WATANABE and T. TAKANO, *J. Pharm. Soc. Japan* **78**, 882 (1958).
[6] T. TAKANO, *J. Pharm. Soc. Japan* **78**, 885 (1958).
[7] T. ROBINSON, in *The Organic Constituents of Higher Plants*, p. 48, Burgess, Minneapolis (1967).

RESULTS AND DISCUSSION

A concentrate of the bitter and cathartic materials in oil-free safflower meal was made by extraction with MeOH and EtOAc. A dialysate produced a precipitate (yield 1%) which, upon recrystallization from ethanol, yielded a pure white powder, m.p. 167–170°, $[\alpha]_D^{27}$ −64·3 (EtOH). This gave a positive phenol–sulfuric acid test for sugar[8] and both acidic and β-glucosidase hydrolyses yielded a sugar identified (TLC) as glucose. The aglycone is a white powder, m.p. 57–60°, $[\alpha]_D^{27}$ −32·1 ($CHCl_3$). The UV spectrum, λ_{max} (EtOH) 279 nm, of the glucoside did not shift upon addition of NaOH indicating no free phenolic group was present. However, a shift to λ_{max} 300 nm was observed upon addition of NaOH to the aglycone. A negative Gibbs test[9] on the aglycone showed the position para to the phenolic to be substituted. A sharp band at 1765 cm^{-1} in its IR spectrum indicated the presence of a 5-membered lactone ring. The MS showed a parent peak at *m/e* 388 and a molecular formula of $C_{21}H_{24}O_7$ was established by high resolution MS.

As in the proton magnetic resonance (PMR) investigation of matairesinol (IV),[1] deuterated benzene was found to be the most useful solvent for the aglycone as it gave the best separation of the aromatic proton peaks. Two sharp peaks at δ = 3·45 (6 H) and 3·31 (3 H) indicated the presence of three aromatic methoxyl groups. A broad peak at δ = 5·51 (1 H) could be assigned to an aromatic hydroxyl group both on the basis of its position and breadth and the upfield shift which occurred when the temperature was raised. The region from δ = 6·40 to 7·00 contained several overlapping multiplets representing six aromatic hydrogens. Comparison of the PMR spectra of the aglycone and its derivatives with the spectrum obtained from authentic arctigenin (V) in the same solvent showed that the compounds, while very similar, differed in the lactone region.

Acetylation of the aglycone under mild conditions gave a monoacetate. The significant part of its IR spectrum was the broad OH band appearing at 3460 cm^{-1} indicating an unacetylated hydroxyl group. More severe conditions produced a diacetate. The diacetate IR spectrum had no OH band but, in addition to the 1770 cm^{-1} phenolic acetate and butyrolactone band, the characteristic aliphatic acetate band appeared at 1745 cm^{-1}.

PMR analyses of the lactone regions of the aglycone and the diacetate derivative of the aglycone gave conclusive evidence that the aliphatic hydroxyl was located on C-2 of the lactone. A complex series of peaks in the spectrum of the aglycone in the region of δ = 2·20–3·90, quite different from those observed in arctigenin (V), represented 8·5 protons in addition to those of the three methoxyl groups. The excess one-half proton is not unexpected in a total integral over such a wide spectral region. An '*AB*' quartet occurred at δ = 2·91 (2 H, J = 14 Hz) which could reasonably be assigned to a benzylic methylene group α to a nonprotonated asymmetric carbon by comparison with the arctigenin spectrum in which one methylene resonance occurs at δ = 2·80. It seemed reasonable to assume that the C-2 proton of arctigenin had been replaced by the second hydroxyl group. This assignment was confirmed by the presence of a doublet split '*AB*' quartet at δ = 3·67 (2 H, J_{gem} = 9 Hz, J_{vic} = 7 Hz) which can be assigned to the methylene protons of C-4 also by analogy with the spectra of arctigenin (δ = 3·65). The vicinal coupling to these protons proved that the hydroxyl could not be located on C-3.

Acetylation of both hydroxyls caused selective shifts that virtually eliminated the overlapping of peaks in the aromatic and lactone regions which limited the utility of the

[8] M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REHERS and F. SMITH, *Analyt. Chem.* **28**, 350 (1956).
[9] F. E. KING, T. J. KING and L. C. MANNING, *J. Chem. Soc.* 563 (1957).

spectra of the aglycone and its monoacetate for purposes of structure elucidation. The spectrum of the diacetate in benzene-d_6 at 70° showed the presence of six protons distributed on two nonfused aromatic rings in a 1,3,4 pattern, as in the case of arctigenin. The complete analysis of the complex multiplet arising from the methine proton centered at $\delta = 2{\cdot}59$ confirms the earlier assignment of the aliphatic hydroxyl to C-2 on the basis of the vicinal couplings to the C-4 protons and to one benzylic methylene pair.

The phenolic monoacetate derivative of the aglycone was dehydrated, creating a double bond between positions 2 and 3 of the lactone as demonstrated by the absence of a C-3 proton resonance and the absence of any vicinal spin–spin coupling to the protons on C-4 and C-5. Hydrogenation under acidic conditions and hydrolysis produced a compound that could be considered a semi-synthetic arctigenin (V). Naturally occurring arctigenin has a *trans* lactone configuration.[10] However, hydrogenation of a double bond would give the *cis* isomer. As expected, minor differences were observed between their PMR spectra. The steric effects of the lactone of the conversion product of 2-hydroxyarctiin posed no problem since its MS was identical to that of authentic arctigenin. The bond cleavages within the lactone rings of the conversion product and the natural arctigenin each produced the same diagnostic peak at *m/e* 194, $C_{10}H_{10}O_4^+$.[11] A fragmentation peak at *m/e* 208 which would have been produced had the glucose been on the alternate ring[11] was not present. Thus, the position of the glucose was elucidated and the complete structure of the cathartic principle in safflower meal established.

EXPERIMENTAL

Extraction and isolation procedures. Oil-free safflower meal was continuously extracted with hot MeOH by percolation for 14 hr. The dried extract was then extracted with hot EtOAc for 6 hr. 1 g of the dried extract per 10 ml of H_2O were blended and dialysed against H_2O for 48 hr. The dialysate was replaced with fresh H_2O and the procedure was repeated $\times 4$. A small amount of NaN_3 was added to prevent bacteriological contamination. The dialysates were combined and upon concentration, precipitates were removed by filtration, combined, and recrystallized from EtOH. The product was examined by TLC SiO_2 using $CHCl_3$–MeOH–H_2O (65:25:4). PMR analysis of the glycoside in DMF-d_7 showed a single anomeric proton at $\delta = 4{\cdot}93$ ($J = 6$ Hz) with a β configuration by analogy with the $\delta = 4{\cdot}94$ ($J = 6$ Hz) anomeric resonance of arctiin (II) in the same solvent.

Acidic and enzymatic glycoside hydrolyses. The glycoside was hydrolyzed with 0·5 N HCl for 2 hr at 100°. The aglycone was extracted with $CHCl_3$ and SiO_2 TLC analysis in several different solvent systems showed it to be pure. The residual aqueous mixture was deionized with Duolite A-4 resin and the sugar identified as glucose on SiO_2 plates developed with PrOH–EtOAc–H_2O (7:2:1), visualized with anisaldehyde spray. The glycoside was incubated with β-glucosidase (2 mg in 1 ml 0·1 M sodium acetate buffer, pH 5·0) for 48 hr at 37°. The aglycone was extracted with $CHCl_3$ and SiO_2 TLC analysis showed the aglycone to be the same as obtained by acid hydrolysis. The measured mass of the acid hydrolyzed aglycone was 388·1529; calculated mass for $C_{21}H_{24}O_7$ is 388·1522.

Aglycone monoacetylation. The aglycone was treated with Ac_2O at 37° for 72 hr. Only one product was observed by TLC analysis. The MS MW was 430. PMR (benzene-d_6) showed one phenolic acetate group; $\delta = 1{\cdot}86$ (3H).

Aglycone diacetylation. The aglycone was heated under reflux at 140° for 2 hr with Ac_2O–KOAc. The residue was taken up in $CHCl_3$, filtered and applied to a 0·5 mm SiO_2 plate which was developed with $CHCl_3$–acetone (9:1). The pertinent band was extracted with $CHCl_3$. The MS MW of the aglycone diacetate was 472. PMR (benzene-d_6) showed two acetate groups. $\delta = 1{\cdot}88$ (3H) and $\delta = 1{\cdot}64$ (3H).

Dehydration and reduction of 2-hydroxyarctigenin. 100 mg of the aglycone monoacetate was shaken overnight with 0·5 ml of 10 ml dry pyridine and 0·15 ml $SOCl_2$. The reagents were evaporated and the procedure repeated $\times 2$. The reagents were evaporated and the residue, taken up in $CHCl_3$, was applied to a 0·5 mm SiO_2 plate which was developed with $CHCl_3$–acetone (9:1). The dehydration product band was eluted from the SiO_2 with $CHCl_3$ and dried. The MS MW of the dehydrated monoacetate was 412. PMR

[10] W. M. Hearon and W. S. MacGregor, *Chem. Rev.* **55**, 957 (1955).
[11] R. Palter, W. F. Haddon and R. E. Lundin, *Phytochem.* **10**, 1587 (1971).

(benzene-d_6) showed one phenolic acetate; $\delta = 1{\cdot}88$ (3H). Hydrogenation using 5% Pa/C in EtOH–HCl was performed overnight at 78 psi H_2 pressure at 70°. After filtration and solvent evaporation, the residue was applied to a SiO_2 plate which was developed with $CHCl_3$–acetone (9:1). The band having the same R_f value as arctigenin was eluted from the SiO_2, extracted with $CHCl_3$ and dried. The MS MW of the synthesized arctigenin was 372.

Cathartic activity assay. The cathartic activity of 2-hydroxyarctiin was determined according to the method of Masri *et al.*[12] Preliminary evidence indicates 2-hydroxyarctiin is *ca.* 10× as potent a cathartic as castor oil.

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[12] M. S. MASRI, L. A. GOLDBLATT, R. DEEDS and G. O. KOHLER, *J. Pharm. Sci.* **51,** 999 (1962).