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Aloe-emodin glycosides of senna leaf

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A new glucoside has been isolated from senna leaf (*Cassia senna* L.) and shown to be identical with the aloe-emodin-8-mono-β-D-glucoside recently isolated from *Rheum palmatum*. This new quinone glucoside is insoluble in water and is present in only small quantities and therefore unlikely to contribute much to the activity of the drug. Confirmation of the presence of a second, highly water soluble glycoside based on a reduced form of aloe-emodin is given; this glycoside may well be responsible for the reported "synergistic effect" of the non-rhein glycosides of senna leaf.

IN 1961, Crellin, Fairbairn, Friedmann & Ryan published a preliminary report on the presence in senna leaf of two glycosides based on aloe-emodin, one of which had been isolated in sufficiently pure form to indicate it to be an aloe-emodin glucoside. A counter-current method for its purification is now described. Using this method sufficient of the glucoside was obtained to establish its structure. We also obtained further evidence for the presence of the other aloe-emodin glycoside. The term "glucoside" is used for the substance of established structure and "glycoside" for the second substance, or a mixture of both.

Experimental

CHROMATOGRAPHY

Examination of numerous systems showed that the upper phase of n-butanol-ethanol-water (5:1:4), the single phase system ethyl methyl ketone-methanol-water (20:1:5) and the lower phase of water-acetone-benzene (2:1:4) gave the best results. The Rf values for the aloe-emodin glucoside in the three systems were 0·48, 0·66 and 0·33 respectively; in the first two systems the sennosides had very low Rf values (0·22 to 0·05) and in the third system high values (0·85 each). Whatman Paper No. 20, ascending technique and room temperature were used.

PRELIMINARY FRACTIONATION OF THE LEAF COMPONENTS

Moderately fine leaf powder was percolated with chloroform to remove free compounds and pigments. The marc was dried, and extracted with 10 to 12 volumes of methanol by percolation; the percolate was evaporated to dryness *in vacuo* yielding a yellow brown residue containing 9·5% rhein glycosides and 2·5% aloe-emodin glycosides, both calculated as sennosides. Further enrichment of the latter was effected by dissolving the extract in the lower phase of n-butanol-ethanol-water (5:1:4) and extracting about 10 times with equal volumes of the upper phase. Most of the aloe-emodin glycosides passed into the upper phases which on evaporation to dryness *in vacuo* and exhaustion with benzene to remove any newly formed aglycones yielded a solid containing 4·4% aloe-emodin glycosides, and small quantities only of sennosides. This fraction was used for the subsequent counter-current work.

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COUNTER-CURRENT SEPARATION

Batches of 9 g of the solid fraction already referred to were distributed between ethyl methyl ketone-0·05% sodium chloride* in water (4 : 3) on a counter-current machine till 60 transfers had been effected. Paper chromatographic examination showed that the aloe-emodin glucoside had been carried to the end of the train. Contents of the appropriate tubes were removed and evaporated to dryness *in vacuo* to give 3 g of a yellow residue containing 7·92% of aloe-emodin glycosides. This residue was distributed between n-butanol-ethanol-0·05% aqueous sodium chloride solution (5 : 1 : 4) till 60 transfers had been effected. After paper chromatographic examination the contents of appropriate tubes were evaporated to dryness *in vacuo* to yield 1·2 g solid. Further impurities were removed by washing the residue with ethanol-methanol (1 : 1) till the washings were colourless. The residue from 3 batches (200 mg) gave a single spot for aloe-emodin glucoside in the systems already referred to.

The residue (100 mg) was dissolved in 0·6 ml dimethylsulphoxide; carbon tetrachloride (10 ml) was gradually added and the solution was then warmed and filtered. After storage at 5° for a few days, 75 mg of crystals were deposited. These were separated and recrystallised in the same manner and dried *in vacuo*.

PROPERTIES AND IDENTITY OF THE ALOE-EMODIN GLUCOSIDE

The yellow, needle-shaped microcrystals were insoluble in ether and chloroform, almost insoluble in water, sparingly soluble in methanol, ethanol and acetone, more soluble in 70% methanol and very soluble in dimethylsulphoxide.

The glucoside gave an orange red colour with alkali and a bluish red colour with concentrated sulphuric acid. The glucoside had a m.p. of 237–238°; λ max (methanol), 222 mμ ($\log \epsilon = 4{\cdot}52$), 255 m$\mu$ ($\log \epsilon = 4{\cdot}41$), 410 m$\mu$ ($\log \epsilon = 3{\cdot}93$); υ max (KBr): C = 0 (free) 1670 cm^{-1}; C = 0 (chelated) 1624 cm^{-1}; C = C, 1580 cm^{-1}. Paper chromatography (see before); thin-layer chromatography, ethyl acetate-methanol-water (100 : 16·5 : 13·5) Rf = 0·50.

Combustion analysis (after drying over $MgClO_4$ *in vacuo* 100–102°). Found: C, 56·8; H, 4·8; calc. for $C_{21}H_{20}O_{10}$, $\frac{1}{2}H_2O$; C, 57·1; H, 4·8; calc. for $C_{21}H_{20}O_{10}$: C, 58·3; H, 4·6.

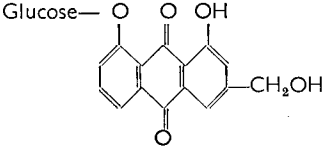
HYDROLYSIS

A known weight of the dried glucoside was hydrolysed in 3N hydrochloric acid and the aglycone separated by extraction with ether, a part of which was evaporated to dryness. The yellow residue was recrystallised from toluene and the crystals sublimed by gently heating in a high vacuum. Examination in several chromatographic systems showed aloe-emodin only was present. The m.p. was 223–224° (lit. 223–224°). Amount present by colorimetric assay in sodium hydroxide [E(1%, 1 cm) for aloe-emodin = 320] was 60·9%.

* The use of water alone causes emulsions.

The aqueous phase was examined chromatographically and shown to contain glucose only; the amount present was estimated by the oxidase method of Hugget & Nixon (1957) giving 37·0% and the *o*-toluidine method of Hultman (1959) giving 36·8%. Anhydrous monoglucoside of aloe-emodin should yield aloe-emodin 60% and glucose 40%.

Our glucoside is identical with the aloe-emodin monoglucoside isolated from *Rheum palmatum* by Hörhammer, Farkas, Wagner & Müller (1964). Comparison with a sample supplied by Prof. Hörhammer showed no depression of melting-point on admixture, and the absorption spectra were also identical. We therefore conclude that our glucoside is aloe-emodin-8-mono-β-D-glucoside.



A SECOND ALOE-EMODIN GLYCOSIDE

A methanolic extract of the leaf was fractionated on a polyamide column (Ultramid K228 BM2, Badische Anilin & Soda Fabrik) by elution with water. The aloe-emodin glucoside was retained on the column but chemical assay (*Analyst*, 1965) of the first fractions of the eluate showed that significant amounts of "non-rhein" glycosides were present along with water-soluble rhein type glycosides. These fractions were hydrolysed with acid, the aglycones extracted into ether and the rhein-type aglycone removed by extraction with aqueous sodium bicarbonate solution. The aglycone remaining in the ether was purified by band chromatography and shown to be a reduced form of aloe-emodin by fluorescence in ultraviolet light, by air oxidation to aloe-emodin in sodium hydroxide solution and by ultraviolet spectrum. The latter, however, differed from that described by Lemli, Dequeker & Cuveele (1964) for a dianthrone of aloe-emodin which they isolated from senna leaf, so that it is not possible at this stage to be certain that both aglycones are derived from the same glycoside. A preliminary report on the presence of a "gluco-aloe-emodin" from senna leaf is given by Romanova & Bankovskii (1965) but no chemical data are given.

Discussion

Since aloe-emodin-8-mono-β-D-glucoside is in the quinone form and is insoluble in water it is most unlikely that it will possess much purgative activity (Fairbairn, 1965). The second aloe-emodin glycoside, on the other hand, is in the reduced form, is highly water soluble and is present to a much greater extent (about ten times the amount of the quinone glucoside). It may therefore be responsible for the synergistic effect reported earlier (Fairbairn & Saleh, 1951). Unfortunately we cannot identify any observed chromatographic spot with this glycoside;

only after hydrolysis is it possible to identify the aglycone. The isolation of the parent glycoside therefore presents considerable difficulty which is not lessened by its high water solubility.

Recently Lemli & Cuveele (1965) and Schmid & Angliker (1965) have isolated two new glycosides (sennosides C and D) from senna leaf, both of which are based on the heterodianthrone of aloe-emodin and rhein. The presence of rhein renders the aglycones soluble in sodium bicarbonate solution so that our previous work on the biological activity of the "non-rhein" glycosides is not affected by the discovery of these new glycosides.

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