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Isolation of a new aloe-emodin dianthrone diglucoside from senna and its potentiating effect on the purgative activity of sennoside A in mice

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Two aloe-emodin dianthrone diglucosides (I and II) were isolated from the leaves of *Cassia angustifolia* Vahl by successive column chromatography with Amberlite XAD-2, silica gel, Polyamide C-200 and Sephadex LH-20. The stereostructures of I and II were elucidated as *trans* and *meso* isomers at 10-10′, respectively, from the patterns of the ultraviolet absorption spectra and circular dichroism curves. This is the first report of isolation of diglucoside I from senna. Despite the lack of purgative activity, diglucoside I exerts a potentiating effect of about 1·3 times on the purgative activity of sennoside A in mice when even 15% is included in the mixture. The difference between I and a third active glycoside based on aloe-emodin is also discussed.

Fairbairn & Saleh (1951a, b) reported that a third active glycoside based on aloe-emodin, i.e. 'nonrhein glycoside', in senna was as strong as sennosides A and B (stereoisomers of rhein dianthrone diglucoside) in purgative activity, and more important, that it exerted a marked synergistic effect, increasing the total activity by about 1·7 times in mice when given as a 15% mixture with sennosides A and B. However Fairbairn & Saleh could not isolate the active glycoside in its pure form.

Recently Kisa et al (1981) demonstrated that sennoside C (rhein-aloe-emodin dianthrone diglucoside), a minor constituent of senna, is nearly equipotent with sennoside A in purgative activity and potentiates its effect by about 1·6 times in mice when given as a 20% mixture with it. In reply to this report, the late Professor Fairbairn, in a private communication, said that he still wondered whether the glycoside based on aloe-emodin dianthrone was also synergistic. We therefore set out to isolate the glycoside based on aloe-emodin dianthrone from senna and re-examine Fairbairn's hypothesis.

MATERIALS AND METHODS

Materials

Senna leaves (*Cassia angustifolia* Vahl) were purchased at the local market. Sennoside A was isolated from them by the methods of Miyamoto et al (1968) and Schmid & Angliker (1965). Aloe-emodin dianthrone was synthesized from aloe-emodin anthrone by the method of Kinget (1967).

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Apparatus

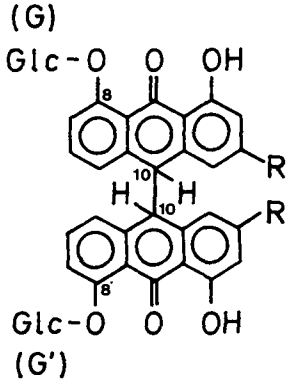
Melting points were measured on a Yanaco micro melting apparatus Model MP-J_3. Ultraviolet absorption spectra were recorded in water or methanol. Circular dichroism spectra were recorded on a JASCO ORD/UV-5 spectrophotometer in dioxane–water (7 : 3). Infrared spectra were recorded on a Shimadzu IR-420 spectrophotometer with a KBr disk. ^{1}H- and ^{13}C-nuclear magnetic resonance spectra were recorded on a JEOL JNM-FX200 spectrometer (Fourier transform) in a mixture of [$^2H_8$]dioxane and [$^2H_2$]water, with the chemical shifts given in δ (ppm) relative to the internal standard, tetramethylsilane. Fast atom bombardment mass spectra were recorded on a JEOL JMS-D300 mass spectrometer. A beam of high velocity xenon atoms was used to generate the ion source.

Thin layer chromatography (TLC)

TLC was conducted using plates precoated with a 0·25 mm thick layer of silica gel 60 (Merck) and the following solvent systems: (1) ethyl acetate–n-propanol–water (6 : 10 : 1) and (2) ethyl acetate–n-propanol–water (4 : 4 : 3) for identification of aloe-emodin dianthrone glycosides; (3) benzene–glacial acetic acid (4 : 1) and (4) n-hexane–benzene–glacial acetic acid (2 : 2 : 1) for identification of aloe-emodin dianthrone. The spots were made visible by ultraviolet irradiation at 366 nm or by heating at 200 °C for 5 min after spraying with 5% potassium hydroxide methanol solution.

Isolation of aloe-emodin dianthrone glycosides

An extract from powdered senna leaves (500 g), kept overnight in 5 litres of 70% methanol, was filtered and the filtrate was concentrated to about 1 litre under vacuum and filtered through Celite (No. 545). It was then washed three times with 700 ml of ether and 500 ml of chloroform, and the aqueous layer concentrated to about 500 ml under vacuum. The aqueous solution was applied to an Amberlite XAD-2 column (600 g, 6 × 34 cm), and eluted successively with 2 litres of water, 4 litres of 20% methanol and about 18 litres of 40% methanol. The last eluate was evaporated to dryness under vacuum, then the residue was extracted twice with 100 ml of methanol under reflux for 10 min, and the methanol solution was evaporated to dryness under vacuum. The residue was dissolved in 50 ml of water, applied to a silica gel 60 (Merck) column (500 g, 6 × 43 cm) and eluted with ethyl acetate–n-propanol–water (10:6:1). The fractions containing aloe-emodin dianthrone glycosides were combined and evaporated to dryness under vacuum, then the residue was dissolved in 50 ml of water and applied to a Polyamide C-200 (Wako) column (250 g, 6 × 38 cm). After the adsorbent had been washed with 2 litres of water, elution with 10% methanol gave a mixture of



Aloe-emodin dianthrone diglucoside
10-10′ *trans*: R = CH_2OH glycoside I
sennoside A 10-10′ *trans* R = COOH
10-10′ *meso*: R = CH_2OH glycoside II
sennoside B 10-10′ *meso* R = COOH

two glycosides (I and II) which appeared as red fluorescent spots similar to those of sennoside A on a thin layer chromatogram developed in solvents (1) and (2) under ultraviolet light. The mixture was dissolved in 10 ml of 70% methanol, applied to a Sephadex LH-20 column (100 g, 3·5 × 26 cm) and eluted with 70% methanol to obtain glycosides I and II. Crude I was repeatedly recrystallized from 70% methanol and yellow crystals were obtained. Crude II was purified further by centrifugal preparative liquid chromatography (Hitachi Model CLC-5) using silica gel KT-2401 (Fuji Gel, 45 g) as the adsorbent and chloroform–methanol (4:1) as the solvent. Repeated recrystallization from ethanol gave yellow crystals of II. A total of 73·0 mg of I and 17·8 mg of II was obtained from 5 kg of senna leaves. The physicochemical properties of glycosides I and II are given in Table 1.

Table 1. Physicochemical properties of glycosides I and II.

	M.p.	Solubility in water	R_F values on silica gel TLC Solvent (1)	Solvent (2)
I	223–225° (decomp.)	Slightly soluble	0·62	0·71
II	205–207° (208° decomp.)	Soluble	0·16	0·61

Hydrolysis of aloe-emodin dianthrone glycosides

A suspension of glycoside I or II in 10 ml of 2·5% sulphuric acid was hydrolysed by heating on a boiling water bath for 15 min. The hydrolysate was extracted with ether, and the ether solution was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was proved to be identical with aloe-emodin dianthrone by TLC and ultraviolet absorption and infrared spectroscopy. The water-soluble fraction of the hydrolysate was neutralized with barium carbonate and then filtered. The filtrate was spotted on Toyo filter paper No. 51 (Toyo Roshi) which was developed twice in n-butanol–pyridine–water (6:4:3). The paper was sprayed with aniline phthalate reagent and then heated at 100 °C for 5 min, which caused a spot identical with glucose to appear.

Biological assay

Female albino mice of the Jcl:ICR strain (CLEA, Japan, Inc., Tokyo) 20 to 35 g, were kept at an ambient temperature of 22 to 25 °C. The purgative assay was carried out by the modified method of Miller & Alexander (1949) based on an 'all-or-none' response in mice, that is, purgation was observed for 8 h after administration of a sample, with the test animals being allowed free access to a diet of MF pellets (Oriental Yeast Co., Ltd, Tokyo), and tap water.

Sennoside A and glycoside I were dissolved in 2% sodium bicarbonate solution and administered orally at a dose of 10 ml kg^{-1}. Preliminary tests showed

that 2% sodium bicarbonate solution had no purgative effect.

The 50% effective dose (ED50) was determined by the probit method.

RESULTS AND DISCUSSION

Fractional purification of aloe-emodin dianthrone glycosides was conducted by examining the hydrolysate of each fraction for aloe-emodin dianthrone by TLC. Despite a number of attempts to separate sufficient quantities for chemical and biological studies, only limited amounts of the glycosides could be obtained.

Structures of aloe-emodin dianthrone glycosides I and II

The infrared spectra of I and II were virtually identical. Their molecular formulae were determined to be $C_{42}H_{42}O_{18}$ from their mass spectra; m/z 857 ($M + Na^+$). Furthermore, both I and II were hydrolysed to aloe-emodin dianthrone and glucose,

Table 2. ^{13}C-nuclear magnetic resonance data of glycosides I, II and sennoside A.

	I	II	Sennoside A (Yamasaki et al 1977)
C-1	161·4	162·5, 161·9	158·7
2	117·9	117·9, 117·6	118·2
3	140·1	141·0, 140·4	135·2
4	124·4	124·4, 123·9	123·9
5	118·3	118·2, 118·0	120·4
6	134·9	135·0, 134·9	135·2
7	114·0	114·2, 114·0	116·5
8	158·7	159·0, 158·8	157·7
9	188·1	188·3, 188·0	186·4
10	56·3	57·5, 56·4	54·0
11	123·4	123·6, 123·5	122·4
12	142·6	143·3, 142·9	138·2
13	150·9	151·1, 150·3	142·3
14	118·8	119·3, 118·8	121·5
15	63·5	63·7, 63·6	165·5
G-1	104·4	104·8, 103·3	103·7
2	74·4	74·3, 73·9	73·5
3	76·1	76·9, 76·5	75·2
4	70·5	70·4	69·7
5	77·8	77·9, 77·8	77·5
6	62·0	62·1	60·7

showing that they are diglucosides of aloe-emodin dianthrone. In ^1H-nuclear magnetic resonance spectra, the signals at δ 4·99 (1 H, d, J = 7·3 Hz) for I and δ 4·94 (1 H, d, J = 7·8 Hz) for II were assignable to the β-anomeric protons of the glucose moiety. The glucosyloxy linkages at C-8 and C-8′ were easily elucidated by comparing the ^{13}C-nuclear magnetic resonance data of I and II with those of sennoside A (Table 2). The stereostructures at the 10-10′ linkage

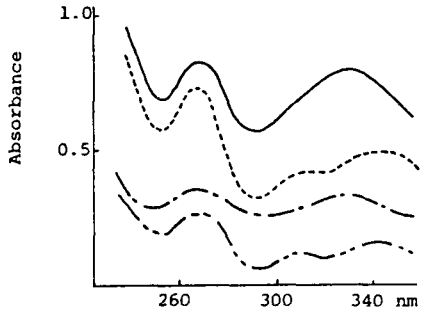


FIG. 1. Ultraviolet spectra of glycoside I, II, sennoside A and B in methanol–water (7:3). —— I, - - - - II, — — — — sennoside A, — — — — — — sennoside B.

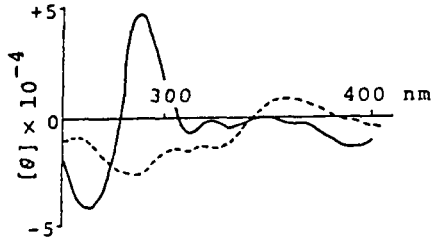


FIG. 2. Circular dichroism curves of glycoside I and II in dioxane–water (7:3). —— I, - - - - II.

of I and II seem to correspond to sennoside A (10-10′ *trans*) and B (10-10′ *meso*), respectively, according to the ultraviolet absorptions of the four compounds (Fig. 1). This was confirmed by the circular dichroism profiles of I and II (Fig. 2). Thus, the structures of I and II were deduced to be 8,8′-diglucosyl aloe-emodin dianthrone (10-10′ *trans*) and (10-10′ *meso*), respectively.

Lemli & Cuveele (1967) reported the isolation of an aloe-emodin dianthrone diglucoside, but did not refer to the stereostructure at 10-10′ linkage. Their compound may be identical with II because the melting point, solubility in water and ultraviolet absorption of II are close to those they described. Therefore, we seem to be the first to have isolated I from senna.

Synergism between sennoside A and aloe-emodin dianthrone diglucoside (10-10′ trans)

Since the amount of II isolated was insufficient for biological investigation, only I, i.e., aloe-emodin dianthrone diglucoside (10-10′ *trans*), was submitted to the animal experiments. Contrary to the suggestion of Fairbairn & Saleh (1951a, b), glycoside I showed no purgative activity even at a dose of 19·7 mg kg^{-1} in mice, whereas an equimolar dose (20 mg kg^{-1}) of sennoside A caused diarrhoea in all mice tested with the ED50 value being estimated as

10·1 mg kg^{-1} (95% confidence limits: 8·9–11·4). The ED50 value of the mixture of sennoside A and I (85 : 15 in mol) was 7·8 mg kg^{-1} (95% confidence limits: 6·8–9·0). This means that I potentiates the purgative effect of sennoside A by only about 1·3 times when present as 15% of the mixture. Thus, the glycoside based on aloe-emodin dianthrone is synergistic to sennoside A as proposed by Fairbairn, but not as active as sennoside A in mice. In a later study, Fairbairn & Moss (1970) concluded that the relative potencies in the aloe-emodin series (e.g. aloe-emodin dianthrone) are much less than in the rhein series (e.g. rhein dianthrone) in mice. Thus, we conclude that the third active glycoside based on aloe-emodin in senna reported by Fairbairn & Saleh (1951a, b) is virtually negligible, though some differences between their results and ours should be taken into account due to variation in the biological assay methods.

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REFERENCES

Fairbairn, J. W., Moss, M. J. R. (1970) J. Pharm. Pharmacol. 22: 584–593

Fairbairn, J. W., Saleh, M. R. I. (1951a) Nature 167: 988

Fairbairn, J. W., Saleh, M. R. I. (1951b) J. Pharm. Pharmacol. 3: 918–925

Kinget, R. (1967) Planta Med. 15: 233–239

Kisa, K., Sasaki, K., Yamauchi, K., Kuwano, S. (1981) Ibid. 42: 302–303

Lemli, J., Cuveele, J. (1967) Pharm. Acta Helv. 42: 37–40

Miller, L. C., Alexander, E. B. (1949) J. Am. Pharm. Assoc. 38: 417–424

Miyamoto, M., Kawashima, K., Imai, S., Shintani, S. (1968) Japanese Published Examined Patent Application No. 43: 16051

Schmid, W., Angliker, E. (1965) Helv. Chim. Acta 48: 1911–1921

Yamasaki, K., Kasai, R., Masaki, Y., Okihara, M., Tanaka, O., Oshio, H., Takagi, S., Yamaki, M., Masuda, K., Nonaka, G., Tsuboi, M., Nishioka, I. (1977) Tetrahedron Lett.: 1231–1234