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ANTI-INFLAMMATORY ACTIVITY OF SAIKOSAPONINS FROM *HETEROMORPHA TRIFOLIATA*

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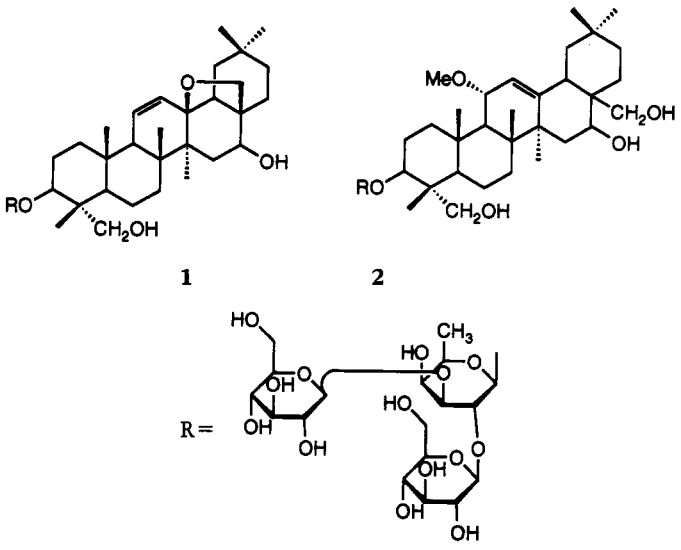
ABSTRACT.—By means of activity-directed chromatographic fractionation using the 12-*O*-tetradecanoylphorbol acetate (TPA)-induced edema test, two saikosaponins were isolated from the MeOH extract of *Heteromorpha trifoliata* leaves. They were identified as 16β,23-dihydroxy-13,28-epoxyolean-11-en-3β-yl-[β-D-glucopyranosyl (1→2)]-[β-D-glucopyranosyl (1→3)]-β-D-fucopyranoside [**1**] and 16β,23,28-trihydroxy-11α-methoxyolean-12-en-3β-yl-[β-D-glucopyranosyl (1→2)]-[β-D-glucopyranosyl (1→3)]-β-D-fucopyranoside [**2**]. Compound **1** showed activity in the TPA and ethylphenylpropiolate (EPP) mouse ear edema and the serotonin paw edema tests, whereas compound **2** was active only in the mouse ear edema model. Both substances had only a slight effect against a carrageenan paw edema model. The anti-inflammatory action of compound **1** was notably decreased by the mRNA and protein synthesis inhibitors actinomycin D and cycloheximide.

Heteromorpha trifoliata Eckl. & Zeyh. (Umbelliferae) is a bush growing in Central and East Africa that is used in traditional medicine for its antimalarial and antiscabies activities (1,2). Two antifungal products, the polyine falcarindiol and the allylbenzene sarisan, have been isolated from a petroleum ether extract of the leaves (1). We have recently examined the anti-inflammatory activity of its MeOH extract, and found it to reduce TPA-induced ear edema and carrageenan-induced paw edema in mice by 88% and 44%, respectively (unpublished data). We report herein the isolation of its active anti-inflammatory principles.

Fractionation of the MeOH extract of *H. trifoliata* on a Sephadex LH-20 column yielded six fractions. The first of these, which contained crude saponins, decreased the TPA-induced edema by 92%. Further purification by chromatographic techniques yielded compounds **1** and **2**. ^{1}H- and ^{13}C-nmr spectral interpretation indicated that these compounds had identical sugar moieties. Acid hydrolysis of the compounds gave glucose and fucose as sugar components. The aglycones in **1** and **2** were identified as saikogenin F and that of saikosaponin B_4, respectively. Because their ^{1}H- and ^{13}C-nmr spectra are identical to those reported in the literature (3,4), compound **1** was characterized as 16β,23-dihydroxy-13,28-epoxyolean-11-en-3β-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl (1→3)]-β-D-fucopyranoside and compound **2** as 16β,23,28-trihydroxy-11α-methoxyolean-12-en-3β-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside. Saponin **1** was identified for the first time in *Buddleja japonica* Hemsl. (Buddlejaceae) (3) and named buddlejasaponin IV. Recently, both saponins have been described in a species of *Bupleurum* (Umbelliferae) (4), a genus related to *Heteromorpha*. The identity and the sequence of sugar units in the trisaccharide attached at C-3 were determined by different spectroscopic techniques.

Both saponins **1** and **2** showed a marked topical activity against TPA-induced edema, with a significant inhibition of 89 and 87% ($p<0.01$, Dunnet's *t*-test), respectively; these values are in the



same range as that obtained for indomethacin (inhibition=89%). When these compounds were administered orally against carrageenan-induced edema, only a slight activity was found 3 h after injection of the phlogistic agent (inhibition=23 and 18%, respectively). This low activity can be explained in terms of gastrointestinal metabolism, as was demonstrated for saikosaponin A, which undergoes a two-step degradation, consisting of a rapid breakdown of the furan ring in the stomach and a further conversion to saikogenin A by the action of intestinal flora (5). When the compounds were subjected to the EPP ear edema test, in which the anti-inflammatory action is much more delayed, only saponin **1** significantly inhibited the edema formation (inhibition=59%, $p<0.01$, Dunnet's *t*-test). This value represents about 3/4 of that obtained for dexamethasone (inhibition=82%). The chemical differences to which one can attribute the different effects of saponins **1** and **2** are the presence of a 13,28-epoxide bridge and an 11,12 double bond in **1** that seem necessary for activity. Since this test serves essentially to determine the activity of glucocorticoids and glucocorticoid-acting substances, saponin **1** may behave like these compounds. Therefore, we tried to demonstrate this by applying a model of glucocorticoid receptor and genomic effects blockade (6). Saponin **1** showed fairly strong anti-inflammatory activity against the serotonin-induced edema, giving a reduction of 48% (significance $p<0.01$, Dunnet's *t*-test). Its anti-inflammatory action was not affected by the blockade of glucocorticoid receptor with progesterone, for a 44% reduction in the edema was still observed. In the presence of actinomycin D and cycloheximide, the anti-edematous effect of saponin **1** decreased to 14% ($p<0.01$, Student's *t*-test), which represents a 71% reduction in the activity (Figure 1).

This is the first time that the anti-inflammatory activity of both saponins has been described, although it should be noted that saponins **1** and **2** differ only in one unit of glucose from saikosaponin A and saikosaponin B_4, respectively. These glycosides, together with other related saikosaponins from *Bupleurum falcatum* L., have previously been studied for their pharmacological properties which include inhibitory action on hepatitis, action on experimental nephritis, sedation, modulation of immune responses, proliferation of cultured hepatoma cells, and anti-inflammatory activity (7,8). Among the saikosaponins, only saikosaponins A and

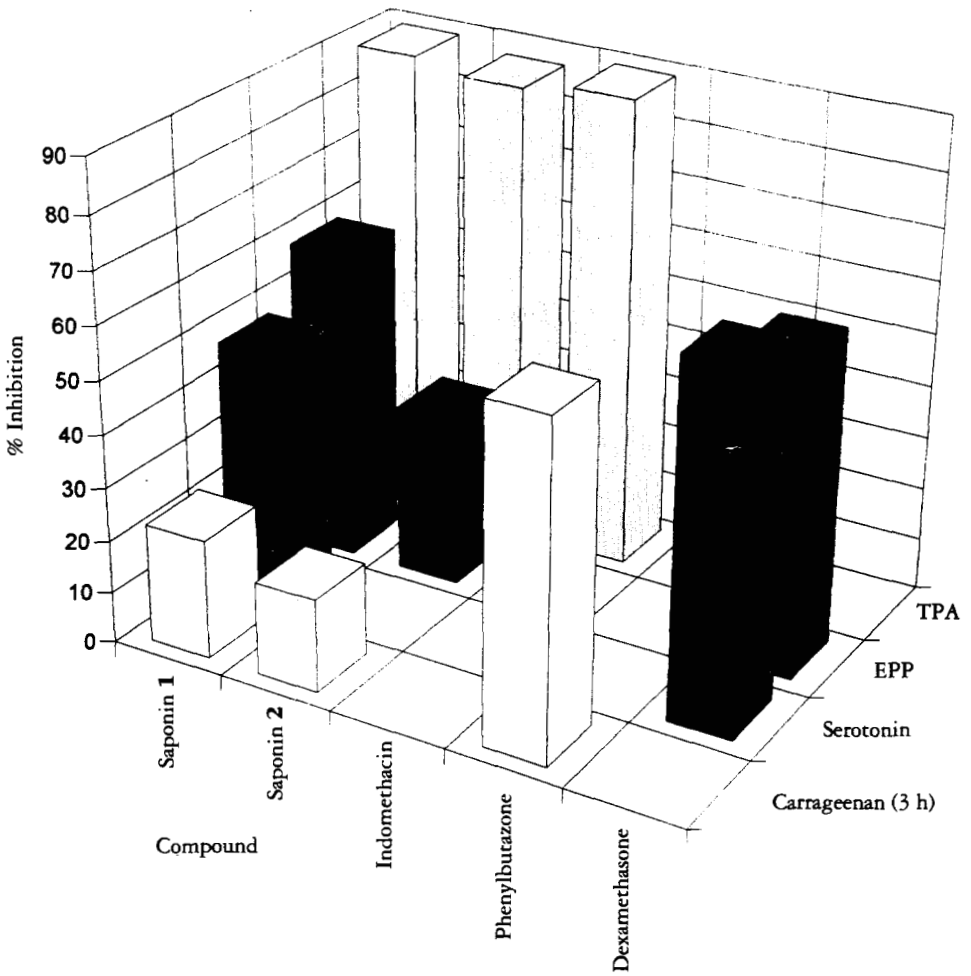


FIGURE 1

D were previously described as active against inflammation in vivo, although they behave in a different way depending on the model assayed. It has been observed that the crude saponin fraction, consisting of saikosaponins A, C, and D, produces a significant decrease in dextran-induced rat paw edema as well as in serotonin- or HOAc-induced vascular permeability responses provoked in the peritoneal cavity of mice, whereas it does not inhibit carrageenan- and HOAc-induced paw edemas (7). More recently it has been shown that saikosaponins A and D, and also C, showed a moderate inhibition of TPA-induced edema (9). It should be noted that another saikosaponin from *B. gibraltaricum* Lam., derived from saikogenin G, has been shown to have an antiedematous effect in the carrageenan test (inhibition=41.4% at 3 h) (10).

Several studies on the mechanism of action of saikosaponins, focusing on PGE_2 production (11), PAF biosynthesis (12), macrophage activation (13), and other processes (14,15), have been carried out. However, the results are frequently inconclusive and therefore new explanations must be sought.

The results obtained suggest that the saikosaponins evaluated interfere with the mechanism of inflammation gener-

ated by TPA, namely an increase in protein kinase C activity, to a larger extent than do the better known saikosaponins A, C, and D. Referring to the mode of action of saponin **1**, it can be stated that due to the large reduction in the effect brought about by adding inhibitors of transcription and translation, the action is probably mediated by the synthesis *ex novo* of inducible proteins, such as lipocortin-1 or certain enzymes counteracting inflammatory mediators, e.g., neutral endopeptidase and angiotensin-converting enzyme (16).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were run in C_5D_5N using a 300 MHz (Varian) instrument. Analytical tlc was carried out on Merck Si gel F_{254} aluminum sheets and Merck RP-8 plates visualized with 1% H_2SO_4/anisaldehyde. The purity of the isolated compounds was checked by hplc-dad on a Merck-Hitachi system with a RP-18 column (5 μm) eluting with a CH_3CN-H_2O-TFA gradient (20:80:0.05→60:40:0.05) for 30 min. The uv detector was set at 210 nm. Acid hydrolysis was carried out with 2 N HCl for 2 h. The tlc of sugars was performed with Si gel eluted with EtOAc-AcOH-MeOH-H_2O (65:20:15:15) and spraying with 0.5% thymol in H_2SO_4-EtOH (5:95).

PLANT MATERIAL.—*Heteromorpha trifoliata* leaves were collected on the Zomba Plateau in Malawi in November 1989. A specimen has been deposited at the National Herbarium of Malawi, Zomba.

EXTRACTION AND ISOLATION.—Air-dried and powdered leaves of *H. trifoliata* were extracted successively with CH_2Cl_2 and MeOH at room temperature. The solvents were removed under reduced pressure. The MeOH extract (17 g) was chromatographed over a Sephadex LH-20 (Pharmacia) column. Six fractions were obtained by eluting with MeOH. The fractions which contained the crude saponins were active in the TPA test (see below) and were combined and rechromatographed over a Lobar LiChroprep RP-18 (Merck) column with MeOH/H_2O mixtures. The fraction eluted with MeOH-H_2O (7:3) yielded compounds **1** (578 mg) and **2** (206 mg).

BIOLOGICAL PROCEDURES.—*Animals.*—Groups of six female Swiss mice weighing 25–30 g were used. All animals were maintained in suitable nutritional and environmental conditions throughout the experiments.

Pharmacological tests.—As previously reported (17).

Carrageenan-induced mouse paw edema.—Saponins were dissolved in EtOH-Tween 80-H_2O (2:2:20, v/v/v) and administered orally at 100 mg/kg (0.50 ml), 1 h before carrageenan injection. A reference group was treated with phenylbutazone (100 mg/kg, p.o.). A control group received the vehicle only.

12-O-Tetradecanoylphorbol acetate (TPA)-induced mouse ear edema.—Saponins were dissolved in 80% aqueous EtOH and were applied topically (0.5 mg/ear), simultaneously with TPA. A reference group was treated with indomethacin (0.5 mg/ear). A control group received the vehicle only.

Ethylphenylpropiolate (EPP)-induced mouse ear edema (18).—Saponins dissolved in 80% aqueous EtOH (0.5 mg/ear) and dexamethasone dissolved in Me_2CO (0.5 mg/ear) were applied topically 16 h before the induction of the ear edema. The left ear (control) received the vehicle (Me_2CO or 80% aqueous EtOH).

Blockage by the anti-glucocorticoid progesterone and m-RNA or protein synthesis inhibitors of vascular permeability caused by serotonin in mice (6).—Progesterone (100 mg/kg) was administered s.c. into the dorsal area 1 h before the treatment with the test compounds, dexamethasone (0.5 mg/kg, standard drug) and saponin **1** (50 mg/kg). The test compounds were injected s.c. into the dorsal area but not near the progesterone site.

Effect of actinomycin D and cycloheximide.—Actinomycin D or cycloheximide, dissolved in physiological saline, was given simultaneously with the test compounds and again 1.5 h later in an attempt to inhibit mRNA or protein synthesis for 3 h after application of the test compounds.

Statistics.—Percentages of edema reduction are expressed by the mean with S.E.M. The Dunnet's *t*-test or Student's *t*-test for unpaired data was used for statistical evaluation.

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