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Active Principles of *Tetradenia riparia;* II. Antispasmodic Activity of 8 (14),15-Sandaracopimaradiene-7α, 18-diol

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Received: October 20, 1986

Abstract: *Tetradenia riparia* is one of the most popular medicinal plants in Rwanda. Previously, several new substances have been isolated from the leaves, including a new diterpenediol, i.e. 8(14),15-sandaracopimaradiene-7α,18-diol. This new diterpenediol exhibits a papaverine-like antispasmodic activity on the contractions of the guinea pig ileum provoked by methacholine, histamine, and barium chloride and on the noradrenaline-induced contractions of the rabbit aorta.

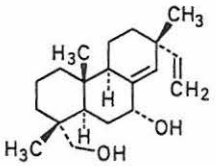

Fig. 1. Structure of 8(14),15-sandaracopimaradiene-7α,18-diol.

Introduction

Tetradenia riparia (Hochst.) Codd (Lamiaceae) is one of the most frequently used medicinal plants in traditional Rwandese medicine (1, 2). Upon biological screening, a substantial antimicrobial (3) and antispasmodic activity (4) was found in the leaves of *T. riparia*. This led to the isolation of the active principle, i.e. 8(14),15-sandaracopimaradiene-7α,18-diol, a new diterpenediol (Figure 1) (5). Several other compounds were isolated from the leaves, including ibozol, 7α-hydroxyroyleanone (6), umuravumbolide (7), 1′,2′-dideacetylboronolide (8), and 8(14),15-sandaracopimaradiene-2α,18-diol (9). Recently, we reported on the antimicrobial activity of the new diterpenediol, i.e. 8(14),15-sandaracopimaradiene-7α,18-diol (10). The antispasmodic effect of the diterpenediol was now investigated by studying its influence on contractions induced by methacholine, histamine and barium chloride in the isolated guinea-pig ileum and by noradrenaline in the isolated rabbit aorta. The antispasmodic effect of the new diterpenediol was compared with that of papaverine (11).

Material and Methods

Chemicals

O-acetyl-β-methylcholine chloride (methacholine) (Schuchardt, München, FRG), histamine (Pharmacy A. De Pannemaeker, Gent, Belgium), barium chloride (Merck), L-noradrenaline bitartrate (noradrenaline) (Sigma Chemical Company, St. Louis, U.S.A.), and papaverine (Pharmacy A. De Pannemaeker, Gent, Belgium).

Isolation of the diterpenediol

8(14),15-sandaracopimaradiene-7α,18-diol was isolated from the chloroform extract of the leaves of *T. riparia* and was purified by chromatography on a silica gel column using a C_6H_6-$CHCl_3$-MeOH gradient and eluted with $CHCl_3$-MeOH (97:3) (5).

Isolated pig ileum

2.5 cm segments of terminal ileum were removed from male or female guinea pigs. The tissues were suspended in 15 ml organ baths (load 5 g) containing Tyrode's solution at 37° C, gassed with 5 % CO_2 in O_2. Isometric contractions were recorded on a Beckman Type R Dynograph recorder. After an equilibration period of one hour, cumulative dose-response curves were obtained with one of the following spasmodics: methacholine (1.10^{-8} M–1.10^{-6} M), histamine (1.10^{-8} M–1.10^{-5} M), or barium chloride (1.10^{-5} M–$2.3.10^{-3}$ M).

Five curves were obtained with an interval of 10 minutes (T_0, T_{10}, T_{20}, T_{30}, T_{40}). Papaverine (1.10^{-5} M, $3.3.10^{-5}$ M, or 1.10^{-4} M) was administered 5 minutes before the third curve (T_{15}) and the diterpenediol ($3.3.10^{-6}$ M, 1.10^{-5} M, or $3.3.10^{-5}$ M) 5 minutes before the fifth curve (T_{35}). In control strips, papaverine and the diterpenediol were not administered. Contractions induced in the presence of papaverine (T_{20}) were expressed in % of the maximal effect of the second curve (T_{10}) and

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those in the presence of the diterpenediol (T_{40}) in % of the maximal effect of the fourth curve (T_{30}). The dose-response curves in the presence of papaverine and the diterpenediol were respectively compared with the third and fifth curve of the control experiments.

Isolated rabbit aorta

The thoracal aorta was removed from male or female rabbits. The aorta was cut in spiral form in segments of 2 cm. The segments were suspended in 15 ml organ baths (load 4 g), containing Krebs solution at 37° C gassed with 5 % CO_2 in O_2. Isometric contractions were recorded on a Beckman Type R Dynograph recorder. After an equilibration time of 90 minutes a cumulative dose-response curve was obtained with noradrenaline (1.10^{-9} M–3.10^{-5} M) (T_0). This was repeated at 90 (T_{90}) and 180 (T_{180}) minutes. Papaverine (2.10^{-4} M, 3.10^{-4} M, or 4.10^{-4} M) was administered 5 minutes before the second curve (T_{85}) and the diterpenediol (2.10^{-4} M, 3.10^{-4} M, or 4.10^{-4} M) was administered 5 minutes before the third curve (T_{175}). In control experiments, papaverine and the diterpenediol were not administered. All contractions were expressed in % of the maximal effect of the first curve. The dose-response curves in the presence of papaverine and the diterpenediol were respectively compared with the second and third curve of the control experiments.

The results are given as mean ± s.e.m.

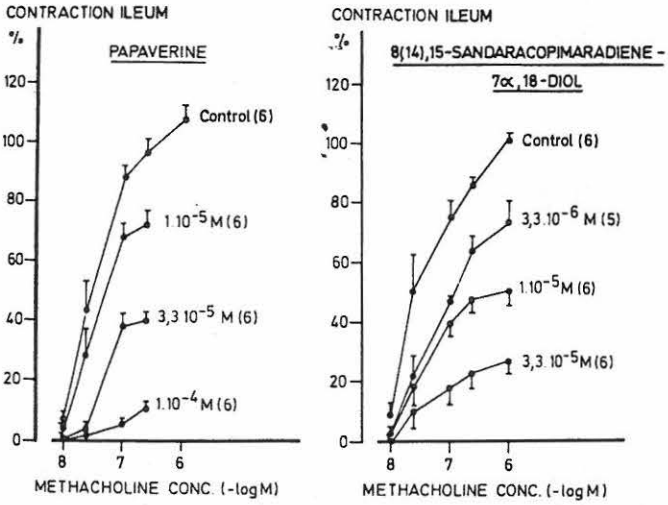

Fig. 2. Effect of increasing concentrations of papaverine (left) and 8(14),15-sandaracopimaradiene-7α,18-diol (right) on the cumulative dose-response curve to methacholine in the guinea-pig ileum. The contractions are expressed in % of the maximal contraction obtained in the same tissue before the administration of the antispasmodic. The bracketed figure indicates the number of experiments.

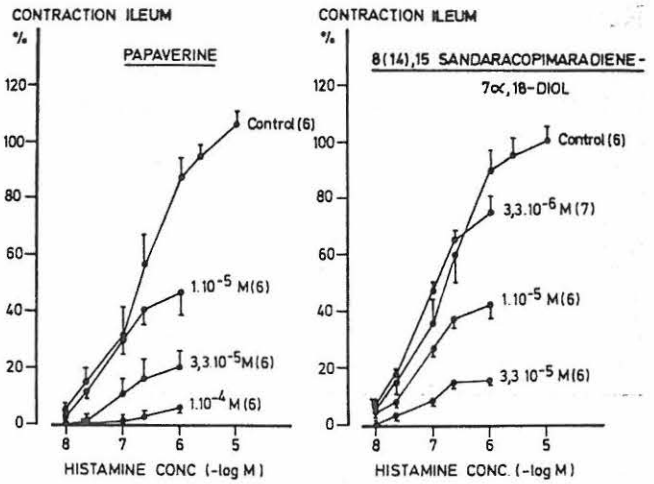

Fig. 3. Effect of increasing concentrations of papaverine (left) and 8(14),15-sandaracopimaradiene-7α,18-diol (right) on the cumulative dose-response curve to histamine in the guinea-pig ileum. The contractions are expressed in % of the maximal contraction obtained in the same tissue before the administration of the antispasmodic. The bracketed figure indicates the number of experiments.

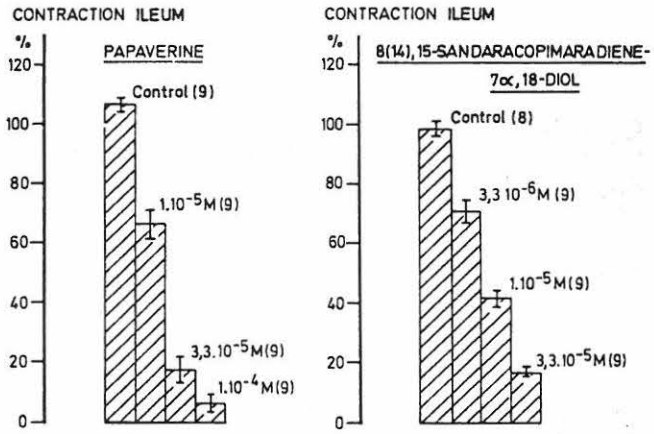

Fig. 4. Effect of increasing concentrations of papaverine (left) and 8(14),15-sandaracopimaradiene-7α,18-diol (right) on the contraction induced by barium chloride (2.3×10^{-3} M) in the guinea-pig ileum. The contractions are expressed in % of the maximal contraction obtained in the same tissue before the administration of the antispasmodic. The bracketed figure indicates the number of experiments.

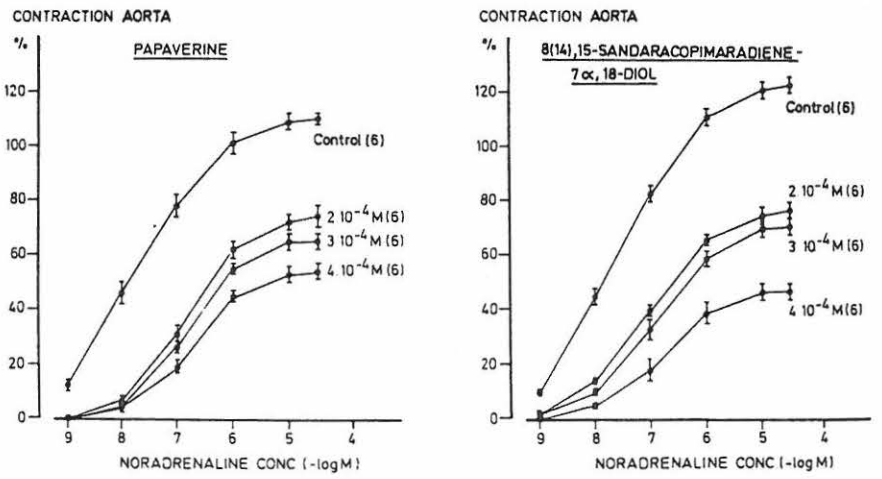

Fig. 5. Effect of increasing concentrations of papaverine (left) and 8(14),15-sandaracopimaradiene-7α,18-diol (right) on the cumulative dose-response curve to noradrenaline in the rabbit aorta. The contractions are expressed in % of the maximal contraction obtained in the same tissue before the administration of the antispasmodic. The bracketed figure indicates the number of experiments.

Results and Discussion

The antispasmodic activity of 8(14),15-sandaracopimaradiene-7α,18-diol compared with that of the known antispasmodic papaverine is presented in Figures 2, 3, 4 and 5.

For the effect on contractions induced by barium chloride, only the influence on the contraction, induced by the highest concentration of barium chloride given, was determined, as the responses induced by lower concentrations were very variable.

The new diterpenediol, 8(14),15-sandaracopimaradiene-7α,18-diol was found to antagonize in a concentration-dependent way the contractions of the guinea-pig ileum induced by methacholine, histamine and barium chloride and the contractions of the rabbit aorta induced by noradrenaline. The potency of the diterpenediol was similar to that of papaverine. The capacity of the diterpenediol to antagonize the contractions induced by 4 different agonists in 2 different tissues, and the similarity of its effect to that of papaverine, indicates that 8(14),15-sandaracopimaradiene-7α,18-diol has non specific smooth muscle relaxing effects.

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Senburiside II, a New Iridoid Glucoside from *Swertia japonica*

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Received: August 1, 1986

Abstract: The structure of senbburiside II, a new iridoid glucoside isolated from *Swertia japonica*, was elucidated to be 7-epi-(di-*m*-hydroxybenzoyl)-loganic acid.

Introduction

In a pervious paper, we reported on the isolation of two new iridoid glucosides, senburiside I (7-epi-(*m*-hydroxybenzoyl)-2'-sinapoyl-loganic acid] (1) and swertiaside (**1**) (2), from *Swertia japonica* Makino (Gentianaceae) (Japanese crude drug name: Sen-Buri). This paper deals with the isolation and structure elucidation of a new iridoid glucoside which we have named senburiside II (**2**).

Results and Discussion

The isolation and purification of this glucoside are described in detail in the Experimental. Senburiside II (**2**), was obtained as a colourless amorphous powder, $C_{30}H_{32}O_{14}$, $[\alpha]_D^{22}$: −88.6° (MeOH). Its UV (230 nm, log ε = 4.42) and IR (1700, 1630 cm^{-1}) absorptions were typical of an iridoidic enol ether system conjugated with a carbonyl group. The ^{1}H-NMR spectrum of **2** showed a signal for an olefinic proton at δ = 7.46 ppm (H-3). Two hemiacetalic protons at δ = 5.44 ppm, (d, J = 4 Hz) and at 4.62 ppm (d, J = 8 Hz), were assigned to H-1 and to the anomeric proton of the β-D-glucopyranosyl moiety, respectively. The doublet at δ = 1.22 ppm (J = 7 Hz) was attributed to the methyl group (C-10) geminal with the H-8 proton. The eight aromatic protons at δ = 7.90, 7.64 ppm (each 1H, J = 8 Hz), 7.78, 7.56 ppm (each 1 H, broad s), 7.52, 7.34 ppm (each 1H, t, J = 8 Hz), and 7.32, 7.08 ppm (each 1H, J = 2 and 8 Hz) were assigned to the H-6", 6"', H-2", 2"', H-5", H-5"', and H-4", 4"' protons of the di-*m*-hydroxybenzoyl moiety, respectively.

Both the ^{1}H-NMR and ^{13}C-NMR spectra of **2** were quite similar to those of **1**, except for the signals arising from the one *m*-hydroxybenzoyl group in **2** (seen Table I). Conventional acetylation of senburiside II (**2**) gave senburiside II pentaacetate (**3**), which was further methylated with diazomethane to give senburiside II methyl ester pentaacetate (**4**). In the ^{1}H-NMR spectrum of **3**, signals of four alcoholic acetoxyl groups appeared at δ = 1.91, 1.99, 2.02 and 2.07 ppm (each 3H, s) and one phenolic acetoxyl group at δ = 2.34 pp (3H, s), while in the spectrum of **4**, one methoxycarbonyl group appeared at δ = 3.64 ppm (s). The mass spectrum of **4** showed fragment ion

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