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SPIGANTHINE, THE CARDIOACTIVE PRINCIPLE OF *SPIGELIA ANTHELMIA*[1]

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ABSTRACT.—Spiganthine [**1**] was isolated as the main cardioactive principle from medicinally used extracts of *Spigelia anthelmia*. Its structure was established by spectroscopic methods. The biological effect of spiganthine is characterized by a delay in contraction development of the heart muscle.

Spigelia anthelmia L. (Loganiaceae), a plant native to Asia and tropical America (2,3), is widely used as an anthelmintic in the folk medicine of Brazil (4), Panama (3), and the Virgin Islands (5), and is used against other diseases (3), and is known by the natives of Surinam as a fish poison (6). In the German Homeopathic Pharmacopoeia, an extract of the aerial parts of *S. anthelmia* is official as a remedy for neuralgic and cardiac disorders (7,8). Previous phytochemical investigations have described the isolation of an alkaloid named "spigeliin" with a still unknown chemical structure (9,10). More recently, a research group has investigated the constituents of the plant's aerial parts and isolated isoquinoline, actinidine, choline, and acylated cholines along with flavonoids and acids derived from the shikimic acid pathway (11).

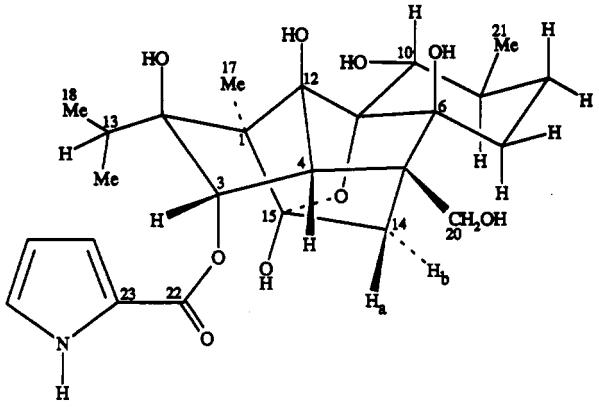
However, these constituents do not explain the significant and very characteristic influence on contraction of isolated heart muscle caused by extracts of *S. anthelmia* (M. Reiter, Technische Universität München, personal communication, 1993) which resemble the effect produced by ryanodine (12,13). The latter compound selectively suppresses the early component of the isometric contraction of guinea-pig cardiac ventricular papillary muscle without necessarily diminishing its late component; thereby it causes a considerable delay in the development of contractions (13). The effect described is explained by the assumption that ryanodine at its intracellular high-affinity binding sites at the terminal cisternae of the sarcoplasmic reticulum junctions keeps the calcium-release channels in the "open state" and thereby prevents the accumulation of calcium (14,15).

We therefore used this effect on the early contraction component of the heart muscle to effect an activity-guided chromatographic purification study in order to isolate the compound(s) responsible for the typical cardioactivity of *S. anthelmia*.

An EtOH extract of the aerial parts of *S. anthelmia* ("Spigelia Urtinktur," prepared according to the prescribed method of the German Homeopathic Pharmacopoeia) was subjected first to cc on Sephadex and Si gel with final separation achieved by hplc on RP-18. Monitoring of the cardioactive effect on the ventricular papillary muscle of guinea-pig hearts (see Experimental) led to the isolation of **1** (4 mg).

Compound **1** was optically active and agreed with an elemental composition of $C_{25}H_{35}NO_9$. The nmr spectra (including HMQC, and especially HMBC) (Figure 1) established the basic structure and nOe studies revealed the relative stereochemistry (Figure 2). Cd studies on **1** and ryanodine (16) suggested the same absolute stereochemistry at C-3.

[1]Part 68 in the series "Constituents of Tropical Medicinal Plants." For part 67, see Achenbach *et al.* (1).



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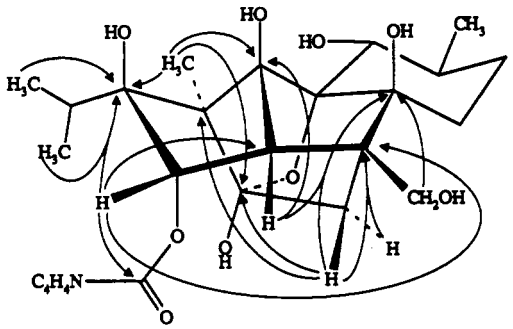

FIGURE 1. Important $^{1}H/^{13}C$ couplings observed in the HMBC nmr spectrum of **1**.

Compound **1**, for which we suggest the name spiganthine, represents a new natural product structurally related to the ryanodines (12,16,17).

Ryanodine-type compounds, which are also well-known for their strong insecticidal activity, have been isolated previously from a few plant species in only the Flacourtiaceae and Lauraceae (17–19). Spiganthine [**1**] can be detected on tlc by its typical orange-red color when sprayed with anisaldehyde reagent (20). The extract of *S. anthelmia* contains some very minor constituents, which, according to their chromatographic behavior, might be structurally related to **1**. They are presently under investigation.

Pharmacological experiments with

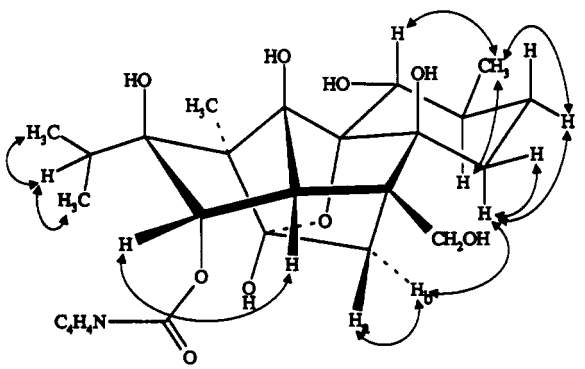

FIGURE 2. Major nOes observed in **1**.

pure spiganthine [**1**] revealed a very high efficacy on cardiac muscle (EC_{50}=60 nmol/liter). Spiganthine produced a concentration-dependent delay in the contraction development of guinea-pig heart ventricular papillary muscle (Figure 3).

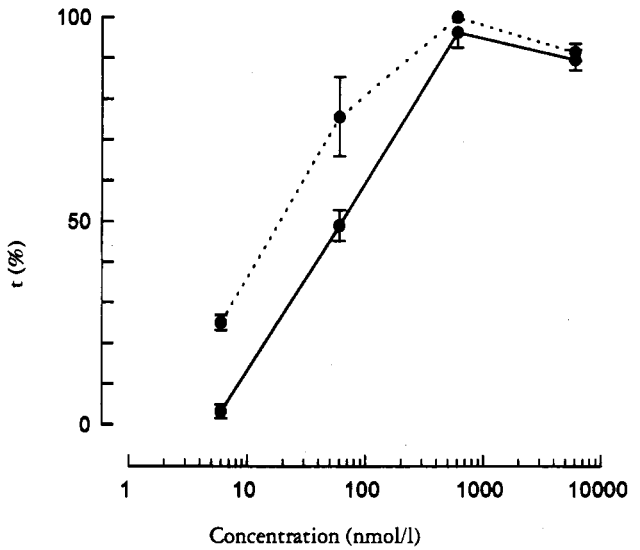

FIGURE 3. Delay in contraction development of heart ventricular papillary muscle by spiganthine [**1**] (—) and ryanodine (·····) at a contraction frequency of 1 Hz. (t: time in % of maximal delay).

The described effect led to the doubling of the period between stimulation and the attainment of 10% of the force of contraction. The half maximal effective concentration at n=3 was 56.7±2.5 nmol/liter. This is slightly higher than the analogous value for ryanodine (16.5±1.5 nmol/liter, n=3) at the same contraction frequency.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp was measured on a Kofler hot-stage apparatus and is uncorrected. Optical rotation, uv, and cd measurements were made in MeOH solution, the ir spectrum was taken on a KBr disk. Nmr spectra were conducted in CD_3OD at 360 MHz (for ^1H nmr) and 90.5 MHz (for ^{13}C nmr); inverse heteronuclear correlations were performed with the sequences of Bax and Summers for HMBC (21) and Bax and Subramanian for HMQC (22); nOes were recorded by the ROESY technique using a pulsed spin-lock field with an average field strength $\gamma B_2 \cong 5$ kHz and a total mixing time of 240 msec (23,24). The dcims (NH_3) was measured on a Finnigan-MAT TSQ 70 instrument; unless they are key ions, only masses with $m/z>100$ and intensities≥20% are stated. The hreims was obtained on a Varian-MAT 312 instrument at 70 eV by peak matching.

Tlc was performed on ready-made SIL-20/UV_{254} nanoplates (Macherey-Nagel) in $CHCl_3$-MeOH-H_2O (80:20:1); detection was carried out by uv and with anisaldehyde reagent according to Stahl (20). For cc, Sephadex LH-20 (Pharmacia) was used with MeOH as solvent; mplc was conducted on Si gel 60 (Macherey-Nagel) at ca. 3 bar using a Büchi gradient 687 apparatus with the $CHCl_3$ concentration decreasing from 100% to $CHCl_3$-MeOH (7:3). Hplc was performed on Nucleosil RP-18 (7 μm) (column length 25 cm) with H_2O-MeOH (3:2); detection by uv at 210 and 254 nm.

PLANT MATERIAL.—Two liters of a tincture from *Spigelia anthelmia* (="Spigelia Urtinktur"), produced according to the German Homeopathic Pharmacopoeia (25) using EtOH-H_2O (86:14), was obtained from DHU (=Deutsche Homöopathie-Union), Karlsruhe (batch nos. 02280562 and 03020508).

EXTRACTION AND ISOLATION.—Evaporation *in vacuo* yielded a residue (14 g), which was separated chromatographically by biological monitoring for cardioactivity as follows: cc on Sephadex

LH-20 using MeOH resulted in 7 fractions (fraction Nos. 1–7), with fraction No. 5 showing the most cardioactivity. It was therefore subjected to mplc on Si gel yielding fraction nos. 5.1 to 5.10. Fraction no. 5.4 was purified by hplc and yielded compound **1** (4 mg) as the main cardioactive principle.

Spiganthine [=*4-deoxy-20-hydroxyryanodine*] [**1**].—Colorless crystals from $CHCl_3$-Me_2CO (3:1) (4 mg): mp 158°; tlc R_f 0.55, red-orange with anisaldehyde; $[\alpha]^{23}D$ +30° (*c*=0.1); ir ν max 3400, 2925, 1680 cm^{-1}; uv λ max (log ε) 227 (3.86), 267 (4.10) nm; cd λ (Δε) 225 (−0.47), 245 (+0.10), 275 (+0.12) nm; ^1H nmr δ 0.81 (3H, d, *J*=7 Hz, Me-18), 1.01 (3H, d, *J*=6.5 Hz, Me-21), 1.16 (3H, d, *J*=7 Hz, Me-19), 1.35–1.41 (1H, m, H_{eq}-7) overlapped with 1.38 (3H, s, Me-17), 1.50–1.64 (2H, m, H_{eq}-8 and H_{ax}-8), 1.73 (1H, dd, J_1=13 Hz, J_2=2 Hz, H_b-14), 1.76–1.86 (1H, m, H-9), 2.10 (1H, ddd, $J_1 \cong J_2 \cong$12 Hz, J_3=6 Hz, H_{ax}-7), 2.21 (1H, d, *J*=13 Hz, H_a-14), 2.36 (1H, qq, J_1=J_2=7 Hz, H-13), 3.48 (1H, d, *J*=11 Hz, H_a-20), 3.54 (1H, dd, J_1=8.5 Hz, J_2=2 Hz, H-4), 3.58 (1H, d, *J*=11 Hz, H_b-20), 3.69 (1H, d, *J*=10 Hz, H-10), 5.78 (1H, d, *J*=8.5 Hz, H-3), 6.22 (1H, dd, J_1=4 Hz, J_2=2.5 Hz, H-25), 6.85 (1H, dd, J_1=4 Hz, J_2=1.5 Hz, H-24), 7.02 (1H, dd, J_1=2.5 Hz, J_2=1.5 Hz, H-26); ^{13}C nmr δ 9.7 (C-17), 18.9 and 19.0 (C-18 and C-21), 19.6 (C-19), 28.0 (C-7), 29.8 (C-8), 31.1 (C-13), 35.3 (C-9), 37.1 (C-14), 51.3 (C-5), 52.6 (C-4), 64.0 (C-20), 65.3 (C-1), 72.5 (C-10), 84.9 and 85.0 (C-3 and C-6), 86.81 and 86.85 (C-2 and C-11), 97.9 (C-12), 103.6 (C-15), 110.9 (C-25), 116.9 (C-24), 123.4 (C-23), 125.5 (C-26), 161.8 (C-22); dcims *m/z* $[MH+NH_3]^+$ 511 (100), $[M]^+$ 493 (69), 476 (31), 400 (39), 388 (25), 383 (22), 382 (79), 371 (22), 365 (21), 298 (42), 281 (20); hreims and eims *m/z* $[M-C_3H_7]^+$ 450.1749 (20) (calcd for $C_{22}H_{28}NO_9$, 450.1756), 432 (16), 414 (10), 346 (58), 316 (75), 293 (26), 211 (30), 193 (52), 177 (58), 154 (49), 137 (31), 111 (89), 94 (100).

BIOLOGICAL MONITORING OF CARDIOACTIVITY.—Papillary muscles (diameter of 0.5–0.8 mm) of the right ventricles of guinea pig hearts were mounted in a 50-ml two-chambered organ bath in which internal circulation was achieved by constantly passing gas through the bath solution composed of 95% O_2 and 5% CO_2 at 35° (26). The solution contained (mmol/liter): NaCl 115, KCl 4.7, $CaCl_2$ 3.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, and glucose 10; the pH was 7.4.

The muscles were stimulated at their base through punctate platinum electrodes with 1 msec square wave pulses of an intensity slightly above threshold. Contractions were recorded isometrically by an inductive force transducer connected to an oscilloscope and a pen recorder. The resting force was kept at 4 mN. The experiments were performed at a contraction frequency of 1 Hz after an equilibration period of 1 h.

To evaluate the effect of the active principle on the early contraction component, the time was measured between the electrical stimulation of the muscle and the 10% value of its arising force.

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