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Thiocardenolides II: Synthesis and Pharmacological Evaluation of 3β-Thioacetyl-14β-hydroxy-5β-card-20(22)-enolide

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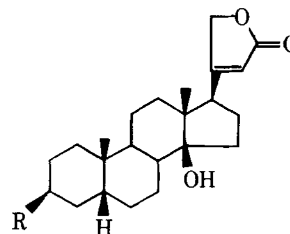
Abstract □ The synthesis of a 3β-thioacetylcardenolide is described. The thioacetate exhibited effects similar to those seen with digitoxigenin acetate on the isolated frog and guinea pig hearts at 1×10^{-7} dilution. In the intact rat heart, the lethal dose was 5 mg/kg for the thioacetate and 2.5 mg/kg for digitoxigenin acetate. The thioacetate inhibited sodium- and potassium-activated adenosine triphosphatase to the same extent as digitoxigenin, but it was somewhat less inhibitory than digitoxigenin acetate.

Keyphrases □ Thiocardenolides—3β-thioacetyl-14β-hydroxy-5β-card-20(22)-enolide synthesized, screened for cardiotonic activity and effect on sodium- and potassium-activated adenosine triphosphatase □ Cardiotonic activity—screened in 3β-thioacetyl-14β-hydroxy-5β-card-20(22)-enolide □ Adenosine triphosphatase, sodium and potassium activated—effect of 3β-thioacetyl-14β-hydroxy-5β-card-20(22)-enolide

The naturally occurring cardioactive steroids are widely used in the therapy of congestive heart failure and atrial arrhythmias and have low therapeutic indexes. There have been numerous efforts to synthesize and evaluate cardenolide derivatives with improved margins of safety, and the literature in this area was reviewed (1). As part of a program to replace oxygens of various cardenolides by sulfur atoms with the aim of separating the therapeutic and toxic actions of the digitalis glycosides, the synthesis and pharmacological actions of the 3β-thioacetyl analog (I) of digitoxigenin (II) are herein reported. A previous paper (2) described the preparation and biological actions of a 3β-thiocyanato analog.



I: R = $SCOCH_3$
II: R = OH
III: R = $OCOCH_3$

EXPERIMENTAL[1]

Chemistry—A solution of 584 mg (1.29 mmoles) of 3-epidigitoxigenin 3-methanesulfonate (2) and 577 mg (5.0 mmoles) of freshly recrystallized (ethanol–water) potassium thioacetate in 10 ml of dimethylformamide (freshly distilled over potassium hydroxide) was heated at 70–90° for 3 hr under dry nitrogen. The reaction mixture was poured onto ice, and the solid product was collected by filtration, dried, dissolved in chloroform,

[1] Melting points were taken on a Fisher-Johns melting-point stage and are uncorrected. UV absorption spectra were determined in 95% ethanol on a Beckman model DK2A recording spectrophotometer. IR absorption spectra were recorded in chloroform on a Beckman model 8 recording spectrophotometer. NMR spectra were determined on a Varian EM 360 spectrometer, using tetramethylsilane as the internal standard and deuterochloroform as the solvent. Microanalyses were conducted by Spang Microanalytical Laboratory, Ann Arbor, Mich. TLC was carried out using Merck silica gel G 254 (0.25-mm thick, analytical plates) or Merck silica gel PF 254 + 366 (0.75-mm thick, preparative plates). Analytical plates were visualized by charring with 5% $Ce(SO_4)_2$ in 6 *N* H_2SO_4; preparative plates were visualized under UV light.

Table I—Adenosine Triphosphatase Inhibition

Compound	Adenosine Triphosphatase Activity, μmoles of Inorganic Phosphate Released/mg of Protein/hr
Control	11.45[a]
I	6.17[b]
II	6.15[b]
III	5.28[b]

[a]Average of four determinations. [b]Average of two determinations.

and filtered. After concentration to dryness, the filtrate was washed with absolute ethanol and filtered to give 378 mg of 3β-thioacetyl-14β-hydroxy-5β-card-20(22)-enolide (I), mp 198–204°.

TLC (1% methanol in chloroform) showed one major spot (R_f 0.3) with two minor products of lower R_f values. Preparative TLC (1% methanol in chloroform) gave 225 mg of a crystalline solid, mp 211–214°. Recrystallization from ethyl acetate–chloroform gave an analytical sample, mp 213–217°; IR: ν_{max} 3600 (OH), 1795 and 1750 (butenolide doublet), 1690 (SC=O), 1610 (C=C), and 730 (CS) cm^{-1}; UV: ν_{max} 219 nm (ϵ = 22,600); NMR: δ 0.88 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 2.33 [3H, s, $CH_3C(=O)S$], 4.06 (1H, broad s, 3-H), 4.95 (2H, broad s, 22-CH_2), and 5.90 (1H, s, 21-CH).

Anal.—Calc. for $C_{25}H_{36}O_4S$: C, 69.41; H, 8.39; S, 7.41. Found: C, 69.44; H, 8.45; S, 7.48.

The IR and NMR spectra indicated that the attack by thioacetate anion on the 3α-methanesulfonate occurred from the β-face of the molecule. The medium absorption band at 730 cm^{-1} (CS) stretch was consistent with the stereochemical assignment of a 3β-thioacetyl *A/B cis*-steroid (3). The epimeric 3β-thioacetyl compound would be expected to absorb at a higher frequency (755–762 cm^{-1}) (3). The broad singlet at δ 4.06 was attributed to the equatorial (α) proton at position 3, indicating that the thioacetyl substituent was axially (β) oriented (4). An axial proton at position 3 should show a peak centered considerably upfield in the δ 3.4 region (4).

Biological Evaluation—Isolated frog hearts (six preparations) were used to evaluate the cardiotonic effect of I according to Straub's method (5). Saline was used as the control, and digitoxigenin acetate (III) was used as the reference standard. The frog hearts were perfused with Frog-Ringer solutions of both compounds, and the changes in chronotropic and inotropic responses were recorded on a smoked-drum kymograph. Analytically pure samples of I were used in this and succeeding experiments.

The action of I on the isolated guinea pig heart was studied using the Langendorff preparation (6). The hearts (six preparations) were perfused with Krebs-Henseleit solutions of I, II, and III, and the inotropic changes were measured on a polygraph recorder[2].

The effect of I on the intact rat heart was studied using three animals anesthetized with pentobarbital (45 mg/kg ip). The cardiac actions were recorded on a polygraph recorder[2]. Saline was used as the control, and digitoxigenin acetate was used as the standard.

Studies of sodium- and potassium-activated adenosine triphosphatase inhibition were carried out using the enzyme obtained from guinea pig brain. The brain of a freshly decapitated guinea pig was homogenized in five volumes of an ice-cold solution (pH 6.8) containing 0.25 *M* sucrose, 5 m*M* histidine, 5 m*M* edetic acid, and 0.2% deoxycholate. The homogenate was centrifuged at 12,000×*g* for 30 min. The supernate was centrifuged at 35,000×*g* for 30 min, and the pellet was suspended in a solution (pH 7.0) containing 0.25 *M* sucrose, 5 m*M* histidine, and 1 m*M* edetic acid. This suspension was centrifuged again at 35,000×*g* for 30 min, and the pellet was resuspended in 20 ml of the same suspending solution. These procedures were carried out at 2°. The quantity of protein in the preparation was assayed by the method of Lowry *et al.* (7).

The enzyme solution (1.0 ml) was diluted to 10.0 ml with the ice-cold suspending solution. To 0.9 ml of the final solution was added 0.1 ml of a 10^{-3} *M* solution of I, II, or III in dimethylformamide and 1.0 ml of a solution containing 50 m*M* tromethamine hydrochloride buffer (pH 7.5), 5 m*M* magnesium chloride, 5 m*M* adenosine triphosphate, 100 m*M* sodium chloride, and 15 m*M* potassium chloride. The control contained the equivalent amount of dimethylformamide. After an 8-min preincubation period, the mixture was incubated at 37° for 20 min. Then 1.0 ml of 15% aqueous ice-cold trichloroacetic acid was added to terminate the reaction.

This mixture was centrifuged for 10 min. The supernate was decanted and mixed with 1.0 ml of a solution prepared by dissolving 400 mg of ferrous sulfate in 10 ml of a 1% ammonium molybdate solution in 1.15 *N* sulfuric acid. The resulting absorbance was read at 700 nm 30 min after the addition of the reagent. Reagent blanks and unincubated tests were also included. The resulting absorbance was converted into micromoles of inorganic phosphate released by comparison with a standard curve (Table I).

[2] Sanborn model 964.

RESULTS AND DISCUSSION

In the isolated frog heart, I elicited a positive inotropic action at 1×10^{-8} dilution as well as at 1×10^{-7} dilution. A negative inotropic effect was seen at dilutions of 5×10^{-6} and lower. On the other hand, digitoxigenin acetate (III) demonstrated a positive inotropic response at 1×10^{-7} dilution and a negative inotropic effect at 5×10^{-5} dilution and lower.

Both I and III had similar actions on the guinea pig heart, causing a prominent inotropic response at 1×10^{-7} dilution and a negative inotropic effect at 5×10^{-5} dilution and lower. Digitoxigenin (II) exhibited a positive inotropic action at dilutions of 1×10^{-7} and 1×10^{-6} and a negative inotropic effect at 1×10^{-5} dilution and lower. At doses of 1 mg/kg, both I and III had a noticeable effect on the lead II ECG of the intact rat heart, as evidenced by decreases in the amplitudes of the T wave and the QRS complex, a prolongation of the PR interval, and a shortened QT segment. A dose of 2.5 mg/kg of III was lethal to the three rats tested; each of those receiving the same dosage of I survived. At a dosage level of 5 mg/kg of I, none of the animals survived.

The results of the interaction of I with sodium- and potassium-activated adenosine triphosphatase indicate that the inhibitory effect is approximately the same as that of digitoxigenin but less than that of digitoxigenin acetate.

It appears that the presence of a 3β-thioacetate moiety on the cardiotonic steroid nucleus does not reduce the cardioactivity associated with digitalis compounds. The results of the lethal dose study on the intact rat heart are encouraging, since they demonstrated that the thioacetyl grouping leads to decreased toxicity. This work is currently being expanded to a study of other thiocardenolides containing thiol and thioacetyl groupings at position 3β to ascertain whether such compounds may have improved margins of safety compared to those currently used in therapeutics.

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