In view of the antituberculous activity of some thiosemicarbazones[9] it was decided to prepare this type of derivative of the mustard aldehydes. The thiosemicarbazones prepared, as well as their crude copper complexes, were completely inactive in a variety of tumor systems.

The condensation of the mustard aldehydes with 4-hydroxycoumarin[10] gave a good yield of the dicumarol analogs IV.

Experimental[11]

Thiosemicarbazones.—A hot solution of 0.91 g. (0.01 mole) of thiosemicarbazide in 30 ml. of water and 2 ml. of glacial acetic acid was added to 0.01 mole of the aldehyde in 25 ml. of ethanol and the mixture was heated for 15 min. Cooling and filtration gave the thiosemicarbazones described in Table I. The copper complexes of these derivatives were prepared by a standard method[12] but were not purified.

Other Aldehyde Derivatives.—A hot solution of 0.01 mole of the aldehyde in a minimum of absolute ethanol was added to a hot solution of 0.01 mole of the hydrazide, hydrazine, or hydrazine salt in a minimum of absolute ethanol[13] and the mixture was refluxed for 15 min. Cooling and filtration gave the compounds listed in Table I.

Dicumarols.—A mixture of 0.015 mole of benzaldehyde mustard and 0.02 mole of 4-hydroxycoumarin in absolute ethanol was refluxed for 15 min. Cooling and filtration gave 5.13 g. (93%) of solid, m.p. 230–231.5°, insoluble in hot ethanol.

Anal. Calcd. for $C_{29}H_{23}Cl_2NO_6$: C, 63.05; H, 4.20; N, 2.72; Cl, 12.84. Found: C, 62.98; H, 4.26; N, 2.62; Cl, 12.92.

In a similar manner *o*-tolualdehyde mustard gave an 84% yield of solid, m.p. 180–181° (for absolute ethanol).

Anal. Calcd. for $C_{30}H_{25}Cl_2NO_6$: C, 63.61; H, 4.45; N, 2.47. Found: C, 63.61; H, 4.59; N, 2.45.

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(10) W. R. Sullivan, C. F. Huebner, M. A. Stahmann, and K. P. Link, *J. Am. Chem. Soc.*, **65**, 2288 (1943).

(11) Analysis by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by Drs. Weiler and Strauss, Oxford, England. All melting points are uncorrected.

(12) B. A. Gingras, R. W. Hornal, and C. H. Bayley, *Canadian J. Chem.*, **38**, 712 (1960).

(13) In cases where 80 ml. of hot ethanol would not dissolve the material the aldehyde solution was added to a suspension.

Microbiological Transformation of Strophanthidin[1]

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During the past few years, reports have appeared on the microbiological transformation of the cardiac aglycones digitoxigenin,[2–10]

gitoxigenin,[3,7,11,12] oleandrigenin,[3] and bufalin.[13] However, no study of the microbiological alteration of the readily available polyfunctional cardenolide, strophanthidin, appears to have been reported to date.

Microbiological transformation products of strophanthidin present attractive targets from several points of view. New structural alterants of the potent cardenolide might be expected to show altered pharmacological properties, and would be of interest in establishing new structure-activity relationships. Secondly, microbiological conversion products might be useful intermediates to new 19-nor[14] or 19-hydroxy[15] steroid hormone analogs. Finally, the nature of the transformations may shed light upon the metabolic degradation of polyfunctional steroids. The present communication reports on the transformation of strophanthidin by the fungus *Chaetomium globosum*.

When strophanthidin (I) was exposed to *C. globosum*, a less polar compound, m.p. 222–224°, was obtained in 8% yield. Analysis afforded figures in good agreement with the formula $C_{23}H_{28}O_5$. The ultraviolet spectrum showed bands at 217 mμ (Δ α,β-lactone) and 241 mμ (Δ α,β-ketone), and the infrared spectrum showed bands at 2.92 μ (OH), 5.62 μ (Δ α,β-lactone), 5.77 μ (—CHO), 6.01 and 6.18 μ (Δ α,β-ketone). On the basis of the foregoing physical constants, the product was characterized as the anhydrostrophanthidone III, and the identity was confirmed by direct comparison with a sample prepared by the procedure of Katz.[16]

The mechanism of the conversion of I to III could involve dehydrogenation at C_3 followed by dehydration, or the alternate sequence involving prior dehydration. The microbiological oxidation of 3-hydroxy steroids to the 3-ketones has been well-established,[17] and

(1) This work was supported in part by grants from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

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(3) M. Okada, A. Yamada, and M. Ishidate, *Chem. and Pharm. Bull.*, **8**, 330 (1960).
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(9) Y. Nozaki, *ibid.*, **25**, 559 (1961).
(10) H. Ishii, Y. Nozaki, T. Okumura, and D. Satoh, *J. Pharm. Soc.* (Japan), **81**, 805 (1961).
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(14) *Cf.* G. W. Barber and M. Ehrenstein, *Ann.*, **603**, 89 (1957).
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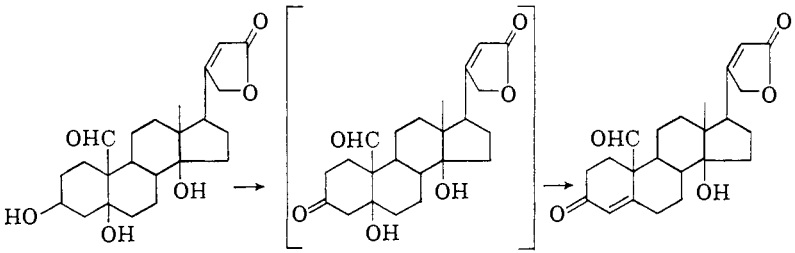

recent examples have been encountered among the cardiac aglycones.[3,7,12] It was deemed desirable to determine whether 5-β-hydroxy-3-ones would undergo dehydration under the mild experimental fermentation conditions. Because 5β-hydroxy-3-one derivatives of strophanthidin have not been reported, coprostan-5β-ol-3-one was used in the dehydration experiments. This ketone was prepared by chromic acid oxidation of coprostan-3β,5β-diol,[18] and was readily dehydrated to Δ^4-cholestenone upon alkaline treatment. When coprostan-3β-ol-3-one was incubated with either autoclaved cells of *C. globosum* or its fermentation filtrates, dehydration to Δ^4-cholestenone likewise proceeded readily. In view of the foregoing, it appears likely that the conversion of I to III involves microbiological oxidation to II, followed by non-enzymatic dehydration to the anhydrostrophanthidone III.

The potency of the anhydrostrophanthidin III was evaluated[19] in etherized cats according to the method of Chen, Chen and Anderson.[20] The lethal dose was 0.5369 $\pm$ 0.0946 mg./kg. (geometric mean in 6 animals). Strophanthidin acetate by this same method showed a lethal dose of 0.1866 $\pm$ 0.0246 mg./kg. and strophanthidin, 0.2570 $\pm$ 0.0154 mg./kg. The concentration used for the above assay was 1–10,000. One animal (not included in the 6 mentioned above) received a continuous infusion of a 1/20,000 concentration. The lethal dose was 2.467 mg./kg. in this animal, which would suggest that III is metabolized rapidly. The order of biological activity of strophanthidin acetate > strophanthidin > anhydrostrophanthidone III is consistent with the hypothesis that the metabolism of strophanthidin in the animal body may proceed *via* a pathway similar to I–III.

(18) P. A. Plattner, H. Heusser, and A. B. Kulkarni, *Helv. Chim. Acta*, **31**, 1885 (1948).

(19) We thank Dr. F. G. Henderson of the Department of Pharmacodynamics of the Lilly Research Laboratories for the pharmacological results reported herein.

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Experimental[21]

Transformation of Strophanthidin (I) into Anhydrostrophanthidone III by *Chaetomium globosum*.—The fermentation medium consisted of corn steep liquor, 0.6%; $NH_4H_2PO_4$, 0.3%; $CaCO_3$, 0.25%; corn oil, 0.22%; yeast extract, 0.25%, and glucose, 1.0%. *C. globosum* was grown in 4.8 l. of this medium (12 2-l. Erlenmeyer flasks); after 24 hr. of incubation at 27° on a rotary shaker, 1.0 g. of strophanthidin in 10 ml. of dimethylformamide was distributed equally among the 12 flasks. The fermentation was allowed to continue for 72 hr.; the culture broth then was filtered and the filtrate extracted with chloroform. The chloroform extract was dried with sodium sulfate and evaporated to dryness to give 1.23 g. of residue. An aliquot of the chloroform extract was chromatographed on Whatman No. 1 paper and developed in a benzene-acetone-water system[22] for 3 hr. The paper chromatogram showed only two spots, one of which corresponded to strophanthidin. The other compound showed an R_f of 0.97 after spraying with 3,5-dinitrobenzoic acid[4]; strophanthidin has an R_f of 0.58 in this system. The residue (1.23 g.) was dissolved in chloroform and chromatographed on 20 g. of silica gel. Elution with chloroform:methanol (98:2) yielded 108 mg. of crude crystals. Two recrystallizations from acetone–petroleum ether gave 81 mg. of crystals, m.p. 222–224°, $\lambda_{max.}^{alc.}$ mμ (ε 24,600), 241 mμ (ε 15,000); $\lambda_{max}^{chf.}$ 2.92 μ, 5.62 μ, 5.76 μ, 6.01 μ, and 6.18 μ.

Anal. Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 72.03; H, 7.26.

The melting point was not depressed upon admixture of a sample of 3,19-dioxo-14-hydroxy-cardodien-(4,20,22)-olid (III) prepared by the procedure of Katz,[16] and the paper chromatographic behavior and solution infrared and ultraviolet spectra were identical with those of the authentic sample.

Coprostan-5β-ol-3-one.—Coprostan-3β,5β-ol[18] (200 mg.) in glacial acetic acid (10 ml.) was treated with chromic anhydride (50 mg.) in acetic acid (2.5 ml.)–water (0.05 ml.). The solution was allowed to stand at room temperature for 3 hr. and then treated with sodium bisulfite solution until green. Water (20 ml.) was added and the solution was extracted with chloroform (three 20 ml. portions). The chloroform extract was washed with water, dilute sodium bicarbonate solution and again with water, and evaporated to dryness. The residue was crystallized from methanol to yield colorless needles (130 mg.), m.p. 160–161°; $[\alpha]^{31}D$ + 51° (*c* 0.74, ethanol).

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 81.24; H, 11.20.

Dehydration of Coprostan-5β-ol-3-one.—A. With Aqueous Methanolic Alkali.—A solution of coprostan-5β-ol-3-one (30 mg.) in methanol (5 ml.) containing aqueous 10% sodium hydroxide (0.5 ml.) was heated for 10 min. on a steam bath, acidified with dilute hydrochloric acid and evaporated to dryness. The residue was crystallized from methanol to yield 15 mg. of Δ^4-cholestenone,

(21) Melting points are uncorrected and were determined in open soft-glass capillaries. Ultraviolet absorption spectra were determined in methanol on a Cary recording spectrophotometer (Model LL MS). Infrared spectra were recorded on a Beckman IR5 double beam infrared recording spectrophotometer. Microanalyses by Dr. S. M. Nagy and associates, Massachusetts Institute of Technology. "Petroleum ether" refers to the fraction of b.p. 60–80°. Silica gel (Mallinckrodt, 2847) was washed with acetone-ether (2:1) and dried at 90–100°. The organism *Chaetomium globosum* was kindly supplied by Professor K. B. Raper, Department of Bacteriology, University of Wisconsin, Madison, Wisconsin.

(22) L. L. Smith, T. Foell, R. De Maio, and M. Halwer, *J. Am. Pharm. Assoc.*, **48**, 528 (1959).

m.p. 79–80°, undepressed by admixture of an authentic sample; infrared spectrum in chloroform superimposable on that of the authentic sample.

B. With Autoclaved *C. globosum* or its Fermentation Filtrates.—*C. globosum* was grown in two 250 ml. Erlenmeyer flasks containing 50 ml. of the medium. After 72 hr. at 27° on a rotary shaker, one flask was autoclaved for 20 min. at 1.05 kg./cm.^{2} and 120°; the other flask was filtered under sterile conditions to remove the mycelia. Coprostan-5β-ol-3-one (10 mg.) in 0.3 ml. of dimethylformamide was then added to each of the autoclaved flasks and the flask containing only the filtrate. The flasks were again placed on the rotary shaker. Chloroform extracts of samples taken at 24, 48 and 72 hr. after steroid addition were chromatographed by the thin layer silica gel plate method,[23] using chloroform as the mobile phase. After spraying with 2,4-dinitrophenylhydrazine (0.4% in 2 *N* HCl) each of the mixtures showed a spot corresponding in mobility to that of Δ^4-cholestenone.

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New Benzomorphan Analgetics

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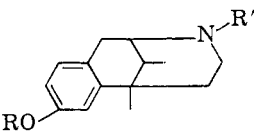
The analgetic phenazocine (I)[1] shows a partial separation of analgesia and addiction liability in both monkeys[2] and humans.[3] Certain other benzomorphans with aralkyl or alkyl groups on the nitrogen seem to extend this separation of effects in animals[4] and some of these aralkyl derivatives are reported in this communication. The compounds prepared and a partial report of their test data are summarized in Table I, with reference to the formula



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(2) G. A. Deneau, D. A. McCarthy and M. H. Seevers, Addendum 1 of Minutes, 20th Meeting of Committee on Drug Addiction and Narcotics, p. 13, Jan. 10–11, 1959, Washington, D. C.

(3) H. F. Fraser and H. Isbell, Minutes of 20th Meeting of Committee on Drug Addiction and Narcotics, Addendum 3, p. 1, Jan. 10–11, 1959, Washington, D. C.

(4) Except for phenazocine, these compounds have not been tested in man.