(2.2 equiv.). After 48 hr. at 22° no phthalic acid had separated and the oxide **5** was recovered unchanged.

(b) A solution of 200 mg. of **5** in 2.5 ml. of chloroform (ordinary) was treated with 2.5 ml. of an ethereal solution of monoperphthalic acid (2.2 equiv.) at 22° for 48 hr., when 1 equivalent of reagent had been consumed. The recovered product solidified readily and crystallization from acetone gave 48 mg. of needles, m.p. 110–115.5°, α_D +12° Chf. Further crystallization from methanol gave needles, m.p. 117–118°, and a mixture with authentic 8α,14α-oxidocholestane-3β,7α-diol 3-acetate (m.p. 122–123°) melted at 117–119°.

Action of Chloroform on the Oxide 5.—(a) A solution of 200 mg. of **5** and 100 mg. of benzoic acid in 5 ml. of pure, dry chloroform was let stand at 22° for 48 hr. Unchanged starting material was recovered.

(b) The experiment was repeated but a drop of water was added. The result was the same.

(c) Chloroform (5 ml.) was shaken with 10% sulfuric acid (3 ml.) and the organic solvent was separated and dried for 10 min. over sodium sulfate. A solution of 200 mg. of the oxide **5** in this sample of chloroform was let stand at 22° for 48 hr. Evaporation of a washed and dried chloroform–ether extract left an oily residue which gave a positive tetranitromethane test and an infrared spectrum showing a band at 2.77 μ. The oil was let stand overnight in a mixture of 2 ml. each of acetic anhydride and pyridine. After recovery of product in the usual way it was adsorbed from petroleum ether onto 5 g. of alumina. Elution with petroleum ether–benzene (9:1) afforded 60 mg. of crystals which on crystallization from ether–methanol gave needles (15 mg.), m.p. 98–100°, identified as a mixture of $\Delta^{7,9(11)}$- and $\Delta^{7,14}$-cholestadiene-3β-ol acetate from spectrographic data: λ^{EtOH} 242 mμ (9,700), shoulders at 235 mμ (8,650) and 250 mμ (6,770); λ^{CS_2} 5.77, 8.08, 9.75 μ. Further elution with petroleum ether–benzene (4:1 and 2:1) gave about 60 mg. of oily product which crystallized from acetone to give 20 mg. of prisms, m.p. 128–131°. A further crystallization from acetone gave material (13 mg.) of m.p. 130–134.5°, α_D −3.8° Chf, tetranitromethane test positive, λ^{EtOH} 207 mμ (11,200). A mixture with authentic $\Delta^{8(14)}$-cholestene-3β,7α-diol diacetate, m.p. 138–140°, melted at 130–137°.

Anal. Calcd. for $C_{31}H_{50}O_4$ (486.71): C, 76.50; H, 10.36. Found: C, 77.12; H, 10.33.

Titration of Acid-treated Chloroform.—A 100-ml. portion of chloroform was shaken with 10% sulfuric acid (30 ml.), dried over sodium sulfate for 10 min., and evaporated. A solution of the residue in 3 ml. of water was treated with potassium iodide–iodate–starch solution and the iodine liberated was titrated with 0.01 *N* thiosulfate solution; 0.4 ml. was consumed, which is equivalent to 0.2 mg. of sulfuric acid.

CAMBRIDGE 38, MASS.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Anhydro Derivatives of Strophanthidin

BY LOUIS F. FIESER AND TOSHIO GOTO[1]

RECEIVED AUGUST 31, 1959

A substance prepared by W. A. Jacobs and co-workers by the reaction of strophanthidin with ethanol containing hydrogen chloride and regarded as the ethylal of 14-anhydrostrophanthidin (**2a**) is in fact the 5-anhydro derivative **2b**. The substance now identified as 5-anhydrostrophanthidin (**3b**) permits evaluation of the effect on cardiotonic activity of a 5,6-double bond. Assays show that this structural feature decreases but does not abolish activity. The seven-ring structure **8** proposed for trianhydrostrophanthidin in 1936 is supported by the nuclear magnetic resonance spectrum of the compound.

In the course of W. A. Jacob's classical investigations of strophanthidin (**1**) Jacobs and Hoffmann[2] found that when dihydrostrophanthidin[3] is refluxed with hydrochloric acid in approximately 50% aqueous methanol one of the two tertiary hydroxyl groups is eliminated with formation of monoanhydrodihydrostrophanthidin. The observation proved important because hydroxylation of the anhydro linkage and oxidation established that the double bond is in a five-membered ring.[4] The degradation also showed that the hydroxyl group at C_{14} is eliminated more readily than that at C_5. Jacobs and Collins[5] found that a solution of strophanthidin in absolute ethanol containing 10% of dry hydrogen chloride on standing at 25° deposits crystals of a monoanhydrostrophanthidin ethylal and, in view of the evidence cited, Jacobs and Elderfield[6] assumed this also to be a 14-anhydro derivative and formulated it as **2a**. The monoanhydrostrophanthidin obtained on hydrolysis of the ethylal was thus formulated as in **3a**.

We noticed that whereas conversion of dihydrostrophanthidin (α_D +35° MeOH) to the 14-anhydro derivative (α_D +48.5° Py) is attended with little change in rotation, conversion of strophanthidin (α_D +43° Al) to the monoanhydride (α_D −145° Al) is marked by a very large levorotatory shift. The strong levorotation of both the monoanhydride and its ethylal (α_D −50° Chf) suggested that they are the Δ^5-derivatives **2b** and **3b**. If the 14β-hydroxyl group is retained, formation of the iso compound **4** should be possible, and indeed Jacobs and Elderfield found that the monoanhydrostrophanthidin is isomerized by base. Convinced that the double bond is at the 14,15-position, they interpreted the isomerization as involving either migration of the double bond in the lactone ring into conjugation with the anhydro linkage or migration of both double bonds to produce a $\Delta^{15,17}$-diene. Actually their isomonoanhydrostrophanthidin is not a conjugated diene, and the ultraviolet and infrared spectra are fully consistent with its formulation as the iso compound **4**. The preferential elimination of the 5-hydroxyl group in the reaction of strophanthidin in anhydrous ethanol indicates that the elimination is coupled with the closing of the 3β,19-oxide bridge. A model of the hypothetical ethylal of strophanthidin shows that rings A and B are both forced to assume the boat conformation and hence that the molecule is under considerable strain. However, the 5β-hydroxyl and the 6α-hydrogen are in the *trans* antiparallel orientation favorable for elimination, and formation of a 5,6-double bond ma-

(1) Recipient of a Fulbright travel grant on leave from Nagoya University, Nagoya, Japan.

(2) W. A. Jacobs and A. Hoffmann, *J. Biol. Chem.*, **74**, 791 (1927).

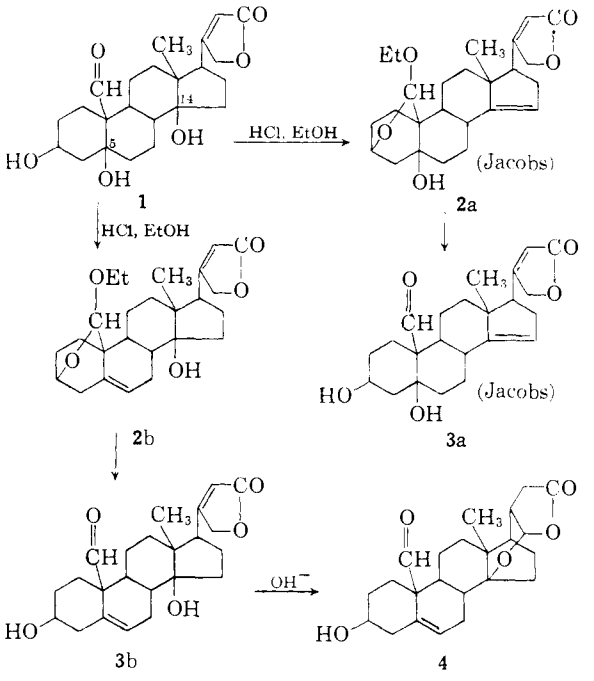
(3) W. A. Jacobs and M. Heidelberger, *ibid.*, **54**, 253 (1922).

(4) W. A. Jacobs and R. C. Elderfield, *ibid.*, **108**, 693 (1935).

(5) W. A. Jacobs and A. M. Collins, *ibid.*, **59**, 713 (1924).

(6) W. A. Jacobs and R. C. Elderfield, *ibid.*, **108**, 693 (1935).

terially relieves the strain. Thus ethylal formation provides the driving force for the specific elimination at C_5.



1 2a 2b 3a 3b 4

With recognition that the monoanhydrostrophanthidin of Jacobs and Collins is actually the Δ^5-derivative, the substance acquires new interest. According to a recent analysis of structural features which contribute in a practical sense to cardiotonic potency,[7] the 14β-hydroxyl and 3β-hydroxyl groups and the unsaturated lactone ring are essential to cardiac activity, and the 19-aldehyde and 5β-hydroxyl groups of strophanthidin, though not essential, contribute significantly to high potency. A 4,5-double bond is about as favorable as a 5β-hydroxyl group, but natural aglycones containing a 5,6-double bond have not been known and information of the effect of such unsaturation is lacking. If unsaturation at this position should prove favorable, a Δ^5-steroid might offer a simpler target for synthesis than the corresponding 5β-alcohol.

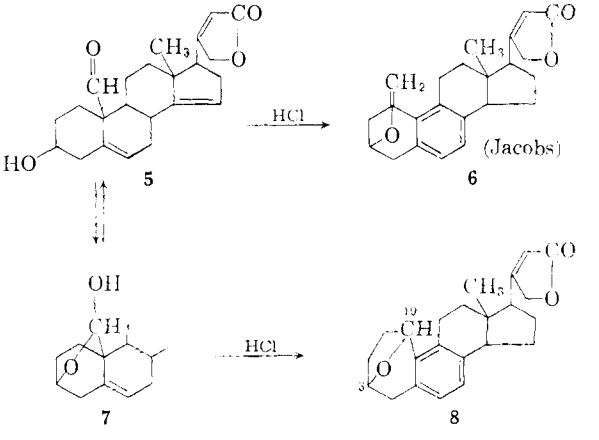
Dr. K. K. Chen and his group at the Lilly Research Laboratories kindly assayed 5-monoanhydrostrophanthidin and its 3-acetate in 10 cats each and reported that "both compounds have digitalis-like action as judged by the characteristic heart rate changes." The free aglycone was assayed by the usual procedure[8] and the less soluble acetate was assayed by the alternative procedure of Chen, Robbins and Worth.[9] The assay results and comparative values for strophanthidin[9] and its acetate[10] are

	Lethal dose, mg./kg.
Strophanthidin	0.3250 ± 0.0232
3-Acetate	0.1866 ± .0246
5-Monoanhydrostrophanthidin	1.930 ± .2244
3-Acetate	1.869 ± .1387

The very marked decrease in potency attending elimination of the 5β-hydroxyl group indicates that the 5,6-double bond is distinctly unfavorable to cardiac activity. Acetylation of the anhydro derivative does not increase potency as does acetylation of strophanthidin.

Since completion of our work, Polonia, Kuritzkes Jäger and Reichstein[11] have established that sysmalogenin, derived from a natural glycoside, is in fact 5-anhydroperiplogenin and hence differs from 5-monoanhydrostrophanthidin only in having a methyl group at C_{10} in place of the aldehyde function. An orienting assay (K. K. Chen) in one cat indicated the mean lethal dose of 2.770 mg./kg.,[12] or 1.4 times the value for the corresponding 10-aldehyde reported above. The accurately determined L_D values for the 10-methyl and 10-aldehydo D-cymarosides and D-glucosides indicate the ratios 1.40 and 1.37.[7] Reichstein and co-workers[11] tentatively suggest that pachygenin may have the structure which we now attribute to monoanhydrostrophanthidin, and the physical constants and assay data on record are not inconsistent with this assignment.

When refluxed in absolute ethanol containing 5% of hydrogen chloride, strophanthidin is converted into an ethylal which on hydrolysis affords dianhydrostrophanthidin[13] (**5**). When stirred with concentrated hydrochloric acid, this substance dissolves with formation of trianhydrostrophanthidin,[14] characterized as lacking the original hydroxyl and aldehydic groups and as having an aromatic ring.[15,16] Evidently a product of rearrangement,



5 6 7 8

trianhydrostrophanthidin, was regarded by both Tschesche and Knick[16] and by Jacobs and Elder-

(7) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp. 800–808.

(8) K. K. Chen, F. G. Henderson and R. C. Anderson, *J. Pharmacol. Exptl. Therap.*, **103**, 420 (1951).

(9) K. K. Chen, E. B. Robbins and H. Worth, *J. Am. Pharm. Assoc.*, **27**, 189 (1938).

(10) F. A. Steldt, R. C. Anderson, N. Maze and K. K. Chen, *Proc. Soc. Exptl. Biol. Med.*, **53**, 198 (1943).

(11) J. Polonia, A. Kuritzkes, Herb. Jäger and T. Reichstein, *Helv. Chim. Acta*, **42**, 1437 (1959).

(12) W. Schmid, H. P. Uehlinger, Ch. Tamm and T. Reichstein, *ibid.*, **42**, 72 (1959).

(13) W. A. Jacobs and A. M. Collins, *J. Biol. Chem.*, **59**, 713 (1924).

(14) W. A. Jacobs and A. M. Collins, *ibid.*, **63**, 123 (1925).

(15) R. C. Elderfield and A. Rothen, *ibid.*, **106**, 71 (1934).

(16) R. Tschesche and H. Knick, *Z. physiol. Chem.*, **229**, 233 (1934).

field[6] as having the formula **6**. One of us[17] suggested that the reaction involves Wagner–Meerwein rearrangement of the intermediate lactal **7** with ring enlargement to **8**. We have now found it possible to distinguish between these two structures by oxidation of trianhydrostrophanthidin to the etio acid and determination of the nuclear magnetic resonance spectrum of the methyl ester, shown in Fig. 1 (40 megacycles per sec., external benzene as reference). In addition to sharp peaks at 217 (f) and

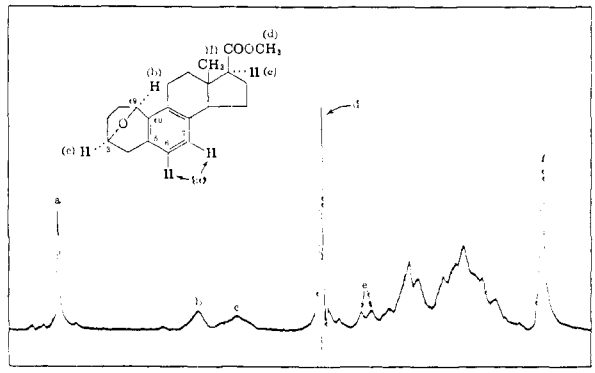


Fig. 1.—N.m.r. spectrum of etianic methyl ester derived from trianhydrostrophanthidin (in $CDCl_3$) in cycles per sec.: a, −20 (aromatic 6-H and 7-H); b, 46 (19α-H); c, 67 (3α-H); d, 108 (ester methyl protons); e, 130 (17α-H?); f, 217 (13-CH_3).

108 (d) cycles per sec. attributable, respectively, to the 13-methyl and ester methyl groups, the spectrum shows an unsplit band (a) at −20 c.p.s. attributable to two equivalent aromatic hydrogens, as required by both formulas. Formula **6** should give rise to a methylene peak, but none was found. Instead, and in support of formula **8**, the spectrum contains low, broad bands of about the same area at 46 (b) and 67 (c) c.p.s. which can be identified with the lone hydrogens at C_{19} and at C_3, respectively, since the aromatic ring can be expected to further shift the proton resonance of the hydrogen on adjacent carbon 19 to lower magnetic field.

Repeated attempts to convert 5-monoanhydrostrophanthidin into the β-D-glucoside led only to recovery of starting material, and we now think that the failure was due to tenacious retention of water of crystallization. At the time, however, it seemed possible that the 5,6-double bond causes the hydroxy aldehyde to exist in the cyclic hemiacetal form. However, comparison of the ultraviolet absorption spectrum with those of other members of the series (Fig. 2) shows that the 5-anhydro compound (curve 3) and its iso derivative (4) exhibit typical aldehydic absorption at 305–308 mμ. In fact these two Δ^5-unsaturated compounds show significantly intensified absorption in this region in comparison to the ring B-saturated strophanthidin (2) and isostrophanthidin (1). The 5,6-double bond thus accentuates absorption by the aldehyde group. The two iso compounds are further distinguished by the fact that one of them (4) shows absorption characteristic of a trisubstituted olefin.

(17) L. F. Fieser, "Natural Products Related to Phenanthrene," 1st ed., Reinhold Publishing Corp., New York, N. Y., 1936.

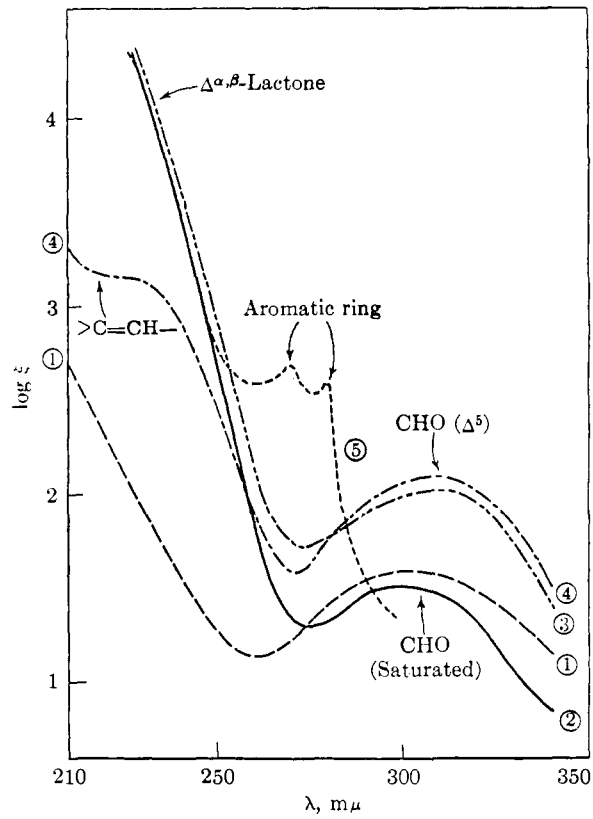


Fig. 2.—Ultraviolet absorption spectra in ethanol: (1) isostrophanthidin, (2) strophanthidin, (3) 5-monoanhydrostrophanthidin, (4) iso-5-anhydrostrophanthidin, (5) trianhydrostrophanthidin.

Acknowledgments.—This work was supported by grants from the National Cancer Institute of the National Institutes of Health (CY1696, Endo), Research Corporation, and the Higgins Fund of Harvard University.

Experimental[18]

3β,19-Oxido-5-monoanhydrostrophanthidin Ethylal (2b).—Prepared according to Jacobs and Collins,[5] the substance was obtained as needles, m.p. 197–200° dec., α_D −43.7° Chf (lit.[5] 223–230°, α_D −50° Chf); λ^{Chf} 5.60$_m$, 5.72$_s$, 6.14$_m$ (unsaturated lactone ring); 9.01$_s$, 10.00$_s$, 11.12$_m$ μ; strong ultraviolet end absorption for the lactone ring, no aldehydic absorption.

5-Monoanhydrostrophanthidin (3b) was prepared as described[5]: needles, m.p. 195–210° (air-dried), α_D −128° Chf (lit.[5] m.p. 223–226°, α_D −145° Al); λ^{Chf} 3.66$_w$ (CHO); 5.60$_m$, 5.74$_s$, 6.14$_m$ (lactone ring); 10.48$_m$, 11.14$_m$, 11.62$_m$ μ; λ^{EtOH} 305mμ (97).

The 3-acetate, described by Jacobs and Collins[5] as melting at 292–294°, melted at 285–290° dec.; λ^{Chf} 3.67$_w$ (C=O); 5.60 (shoulder), 5.75$_s$ (lactone, acetate and CHO); 9.72$_s$, 10.48$_m$, 11.12$_m$, 11.62$_m$ μ.

Iso-5-monoanhydrostrophanthidin, for which Jacobs and Elderfield[6] report m.p. 220–222°, α_D −178° Chf, was obtained as leaflets, m.p. 177–180° dec., α_D −170° Chf, λ^{Chf} 5.64$_s$ (γ-lactone); 3.68$_w$, 5.82$_s$ (CHO); 8.75$_s$, 10.58$_s$, 10.90$_s$ μ (acetal in side chain); λ^{EtOH} 308 mμ (125).

Isostrophanthidin, prepared according to Jacobs and Gustus,[19] who report m.p. 259–261°, α_D +48° Py, melted at 258–262° dec., α_D +35.2° Chf, λ^{Chf} 5.64$_s$ (γ-lactone);

(18) Melting (or decomposition) points uncorrected. Infrared spectra were determined with a Perkin–Elmer model 21 or Infracord model 137 with a sodium chloride prism. Ultraviolet spectra were taken with a Cary model 11M with automatic recorder.

(19) W. A. Jacobs and E. L. Gustus, *J. Biol. Chem.*, **74**, 811 (1927).

3.65_w, 5.82_s (CHO); 8.75_s, 10.60_s, 10.90_s, 11.00_s μ (acetal in side chain); λ^{EtOH} 303 mμ (36).

3β,19-Oxido-5,14-dianhydrostrophanthidin Ethylal.—Jacobs and Collins[14] report m.p. 249–251° with sintering at 238°, α_D −142° Chf. Our sample formed needles, m.p. 229–235° dec., α_D −126° Chf; λ^{Chf} 5.61_s, 5.72_s, 6.13_m (lactone ring); 9.02_s, 9.72_s, 9.87_s, 10.03_s, 11.17_m, 11.60_m μ.

5,14-Dianhydrostrophanthidin is described[14] as m.p. 233–236°, α_D −222° Chf, λ^{EtOH} 303 mμ (126).[15] Our sample formed needles, m.p. 220–223°, α_D −212 Chf; λ^{Chf} 3.67_w, 5.82_s (CHO); 5.60_m, 5.72_s, 6.12_m (lactone ring); 9.57_m, 11.18_m, 11.58_m μ.

Trianhydrostrophanthidin (8), is described by Jacobs and Collins[14] as m.p. 135.5–137.5°, α_D +98° Chf; our sample: prisms, m.p. 133–135.5°, α_D +98.5° Chf; λ^{CS_2} 5.60_s, 5.70_s, 6.12_m (lactone ring); 9.39_s, 9.67_s, 9.81_s, 10.17_s, 11.31_s, 11.66_s, 12.27_s μ (1,2,3,4-tetrasubstituted benzene ring).

Methyl Trianhydrostrophanthidinethianate.—Finely powdered potassium permanganate (1 g.) was added portionwise to a solution of 1 g. of trianhydrostrophanthidin in 60 ml. of acetone with stirring at room temperature. After 30 min. another 0.5-g. portion of permanganate was added and the mixture was stirred for 1 hr. further and filtered. A suspension of the precipitated material in water was acidified with dil. sulfuric acid and extracted with chloroform, and the extract was washed, dried, and evaporated in vacuum. The residue (750 mg.) was treated with diazomethane in ether, and a solution of the crude ester in benzene was adsorbed onto 14 g. of alumina. Benzene eluates gave 240 mg. of crystalline solid which on crystallization from aqueous methanol formed long needles (200 mg.), m.p. 88–89°, α_D +122.3° Chf.

Anal. Calcd. for $C_{27}H_{26}O_8$ (326.42): C, 77.27; H, 8.03. Found: C, 77.17; H, 7.95.

Note.—We are indebted to a referee for the following comment: "Both trianhydrostrophanthidin and 3β,19-oxido-5-monoanhydrostrophanthidin ethylal exhibit *two* maxima in their carbonyl regions of the infrared while these compounds have only *one* carbonyl chromophore, the 5-membered α,β-unsaturated lactone. This fact is deserving of further explanation and is probably another example of Fermi resonance, an interpretation made more attractive by the intensification of the 5.60 μ band of **8** (CS_2) compared to that of **2b** ($CHCl_3$).[20] The presence of this band in several of the other compounds is also rationalized on this basis."[7]

(20) R. N. Jones, T. Ito and C. L. Angell, *Angew. Chem.*, **69**, 645 (1957); P. Wieland, K. Heusler, H. Ueberwasser and A. Wettstein, *Helv. Chim. Acta*, **41**, 74 (1958); P. Yates, N. Yoda, W. Brown and B. Mann, THIS JOURNAL, **80**, 202 (1958); P. Yates and L. L. Williams, *ibid.*, **80**, 5896 (1958); R. Hirschmann, G. A. Bailey, R. Walker and J. M. Chemerda, *ibid.*, **81**, 2822 (1959).

CAMBRIDGE 38, MASS.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Identification of Ketone 104 as 3,4-Secocholestane-6-one-(3α,5α)(3β,4)-dioxide

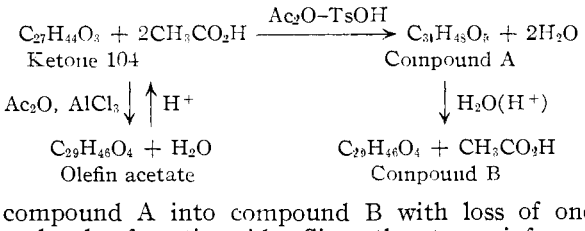
BY LOUIS F. FIESER,[1] TOSHIO GOTO[2] AND BIDYUT K. BHATTACHARYYA[3]

RECEIVED SEPTEMBER 3, 1959

Treatment of ketone 104 with acetic anhydride and aluminum chloride opens one of the two oxide bridges and gives the olefin acetate **4**, in which the double bond and acetate groups are isolated. Hydrogenation and acid hydrolysis gave a product (**6**) shown to be a primary alcohol by oxidation to an aldehyde and then to an acid. Under basic catalysis the hydroxymethyl group of **6** is eliminated as formaldehyde giving the ketone **7**. Wolff–Kishner reduction gave 4-oxacholestane 11, identical with material synthesized from the known **8**. The configuration of ketone 104 was established by synthesis of its desoxo derivative **22** starting with cholestane-3β,4β,5α-ol (**18**). Figure 1, reporting the n.m.r. spectrum, shows the conformation. The unusual reaction leading to ketone 104 is probably related to oxidative rearrangements of a type investigated by W. A. Mosher, and an analogous mechanism is suggested in formulas **29–32**.

Ketone 104,[4] a dioxidic product of the formula $C_{27}H_{44}O_3$ easily obtainable in 4% yield by high-temperature oxidation of cholesterol,[5] is unreactive to the Girard reagent[4] and has previously been characterized by the preparation of the following derivatives[6]: the corresponding alcohol and its acetate, the 2,4-dinitrophenylhydrazone, oxime, lactone, ethylenethioketal, desoxo derivative, and a substance regarded as an "enol acetate." In the first phase of the work now reported, one of us (B.K.B.) effected the transformation of the ketone into a succession of degradation products and derivatives designated by the letters A-J. The results were consistent with the presence of two oxidic bridges, but no known product was encountered and no fully satisfactory interpretation was arrived at. T. Goto later resumed work on the problem and by further characterizations of some of the products with the aid of new and improved instrumental facilities was able to work out the structures and interpretations presented in this paper and in a second one to follow.

Compound A, obtained in good yield by reaction of ketone 104 with acetic anhydride and *p*-toluenesulfonic acid at 25° and decomposition of the reaction mixture with ice and water, bears the relationship to the starting material indicated in the chart. The reaction involves condensation with two molecules of acetic acid, and acid hydrolysis transforms



compound A into compound B with loss of one molecule of acetic acid. Since the strong infrared bands in the fingerprint region[5] disappear on conversion to compound A but reappear on hydrolysis to B, the acetolysis to A involves opening of one oxide bridge and the hydrolysis to B is attended with reformation of this linkage. A fuller study of the infrared spectrum of the supposed "enol acetate," obtained[6] by reaction of ketone 104 with acetic

(1) Paper No. 300.

(2) Recipient of a Fulbright travel grant on leave (1957–1959) from Nagoya University, Nagoya, Japan.

(3) Work done as postdoctoral fellow in 1952–1953.

(4) L. F. Fieser, THIS JOURNAL, **75**, 4395 (1953).

(5) L. F. Fieser, W.-Y. Huang and T. Goto, *ibid.*, **82**, 1688 (1960).

(6) L. F. Fieser and B. K. Bhattacharyya, *ibid.*, **75**, 4418 (1953).