Pechmann condensation of ethyl acetoacetate and phenols does not always yield coumarins as previously reported.

2. Although sulfuric acid causes the condensation of 3,5-dimethylphenol and ethyl acetoacetate to a coumarin, this same reagent results in the formation of a chromone from 4-chloro-3,5-dimethylphenol and ethyl acetoacetate.

3. The structure of the chromone was established by various hydrolytic reactions and by condensation with benzaldehyde.

4. The coumarin expected in the initial condensation was synthesized by nitration of 4,5,7-trimethylcoumarin, followed by reduction and replacement of the amino group by chlorine. The proof that the product was a coumarin and that the chlorine was in the 6-position was determined by ozonolysis to 5-chloro-4,6-dimethyl-2-hydroxyacetophenone.

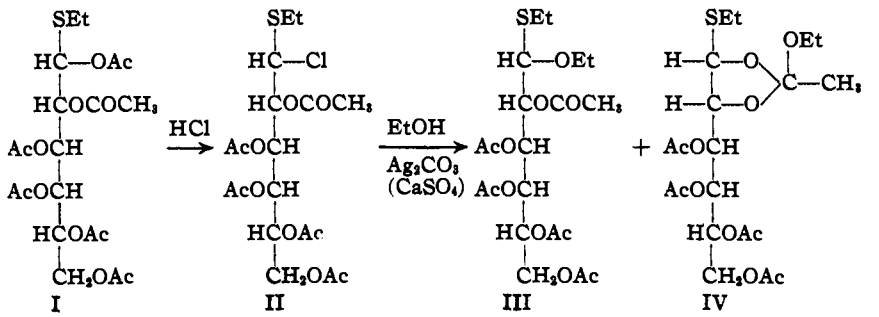
URBANA, ILLINOIS RECEIVED MARCH 2, 1944

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

An Acyclic Sugar Orthoacetate

BY M. L. WOLFROM AND D. I. WEISBLAT

1-Chloro-1-thioethoxy-*aldehydo*-D-galactose pentaacetate (II) has been prepared by the action of a dry ethereal solution of hydrogen chloride upon 1-thioethoxy-*aldehydo*-D-galactose hexaacetate (I).[1] On repeating this procedure in larger quantities, a crude preparation of II was treated with ethanol and silver carbonate. The main reaction product was III, designated D-galactose diethyl monothioacetal pentaacetate and previously synthesized[2] in a similar manner from pure II, prepared by the action of a mixture of acetyl chloride and phosphorus oxychloride upon D-galactose diethyl mercaptal pentaacetate. In addition there was obtained in low yield a product IV, to which is assigned an orthoacetate structure.



The orthoacetate structure of IV, designated 1-thioethoxy-*aldehydo*-D-galactose ethyl 1,2-orthoacetate tetraacetate, is based upon the fact that one acetyl group in the compound was resistant to alkaline hydrolysis but was removable by acid hydrolysis. This fact, among others, has been employed in establishing the orthoacetate structure[3] in the cyclic sugar derivatives. In formula IV, the placement of the sulfur atom and the size of the ring are probable but arbitrary. To our knowledge, this is the first recorded occurrence of an orthoacetate structure in an acyclic sugar derivative.

Experimental

1-Thioethoxy-*aldehydo*-D-galactose Ethyl 1,2-Orthoacetate Tetraacetate.—Crude unrecrystallized 1-chloro-1-thioethoxy-*aldehydo*-D-galactose pentaacetate (m. p. 95–98°, 45 g.), prepared from 1-thioethoxy-*aldehydo*-D-galactose hexaacetate as previously described[1] and thoroughly washed with an ether–petroleum ether (1:1) mixture, was added to a suspension of 25 g. of Drierite (anhydrous calcium sulfate) and 60 g. of silver carbonate in 250 cc. of absolute ethanol. The reaction was then allowed to run at room temperature, under mechanical stirring and protection from atmospheric moisture, for approximately sixteen hours. The inorganic salts were removed by filtration through a bed of Super-cel (Johns-Manville) and the clear filtrate was made just opalescent with water. One crop of crystals was deposited on cooling to ice-box temperature; a second and third crop of crystals were obtained by treating the successive filtrates in a like manner. The first two crops (15–16 g.) were recrystallized from six parts of hot absolute ethanol; m. p. 103–105°, spec. rot. +50° (25°, *c* 4, abs. $CHCl_3$, D line). This product was therefore identified (mixed m. p. with an authentic sample unchanged) as D-galactose diethyl monothioacetal pentaacetate for which the following constants are recorded:[2] m. p. 104–105°, spec. rot. +50° (20°, *c* 3.5, abs. $CHCl_3$, D line). The third crop (6–8 g.) had a melting point of 85–95°. Pure material was obtained after five recrystallizations from four parts of hot methanol; yield 1.5–2.0 g., m. p. 125–126° (mixed m. p. with D-galactose diethyl monothioacetal pentaacetate of m. p. 104–105°, 93°), spec. rot. +54° (24°, *c* 4, abs. $CHCl_3$, D line).

The substance crystallized in transparent, elongated prisms and exhibited properties and solubilities similar to the isomeric D-galactose diethyl monothioacetal pentaacetate, except that it was more sensitive to acidity.

Anal. Calcd. for $C_{10}H_{17}O_6S(CH_3CO)_5$: C, 49.99; H, 6.71; S, 6.67; CH_3CO (4 equiv.), 8.33 cc. 0.1 *N* NaOH per 100 mg.; CH_3CO (5 equiv.), 10.41 cc. Found: C, 49.92; H, 6.73; S, 6.86; CH_3CO (alkaline hydrolysis, Kunz and Hudson[4] method), 8.39 cc.; CH_3CO (acid

(1) M. L. Wolfrom, D. I. Weisblat and A. R. Hanze, THIS JOURNAL, **62**, 3246 (1940).

(2) M. L. Wolfrom and D. I. Weisblat, *ibid.*, **62**, 878 (1940).

(3) K. Freudenberg and E. Braun, *Naturwissenschaften*, **18**, 393 (1930); E. Braun, *Ber.*, **63B**, 1972 (1930); H. G. Bott, W. N. Haworth and R. L. Hirst, *J. Chem. Soc.* 1395 (1930).

(4) A. Kunz and C. S. Hudson, THIS JOURNAL, **48**, 1982 (1926).

hydrolysis, Freudenberg and Harder[5] method), 10.52 cc.

Summary

1. There is reported the synthesis, in crystalline form, of the first acyclic sugar orthoacetate (IV), designated 1-thioethoxy-*aldehydo*-D-galactose ethyl 1,2-orthoacetate tetraacetate.

(5) K. Freudenberg and M. Harder, *Ann.*, **433**, 230 (1923).

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[CONTRIBUTION FROM THE PHYSIOLOGICAL LABORATORIES, CLARK UNIVERSITY]

A Study of the Neutral, Non-Saponifiable Fraction of Ox Bile[1]

BY W. H. PEARLMAN[1a]

A theory[2] postulating a biological conversion of cholesterol to the bile acids has recently received experimental support in an investigation[3] carried out with the aid of deuterium. One might expect to find metabolic products intermediate between cholesterol and the bile acids in bile since it contains these steroids in large amount. Studies of the comparative biochemistry of the bile have already furnished clues as to the nature of the hypothetical intermediates. The bile of elasmobranchs[2] contains a polyhydric alcohol, scymnol ($C_{27}H_{46}O_5$) which is related to cholesterol; it can be readily degraded in the laboratory to cholic acid.[4,5] Other alcohols of an alleged steroid nature have been isolated from the bile of frogs[6] and toads[7,8]; of these, pentahydroxybufostane ($C_{28}H_{50}O_5$) can be oxidized *in vitro* to cholic acid.[7] A mechanism for the biological oxidation of the side-chain of steroids with 27 and 28 carbon atoms has been suggested by Kazuno.[7]

An exploratory examination of the neutral fraction of ox bile failed to reveal the presence of scymnol, etc. On the other hand, five compounds (other than cholesterol[9]) were isolated in small amount. Although the empirical formulas (see Table I) point to a steroid structure, these compounds could not be identified with any known steroids. Structural proof must wait until more material is available.

Compound B, m. p. 192–193°, has an empirical formula identical with that of pregnandiol. Since Compound B is digitonin-precipitable, pregnandiol-3(β),20(α) was suspected to be the isomer in question. This steroid had been prepared by Butenandt and Müller[14] and they reported a melting point of 189–190.5° uncor. Marker, *et al.*,[15] had previously prepared the isomer from pregnandiol but their preparation melted at 182°. This discrepancy in melting points was noted by Butenandt and Müller; they raised a doubt regarding the nature of their own preparation, stating that isomerization of the C-17 side-chain might have occurred since the reduction of the 20-keto group of the intermediary steroid had been carried out in an alkaline medium. The writer prepared pregnandiol-3(β),20(α), following the procedure of Marker, *et al.*,[15] in all respects except that the intermediate 20-acetate of pregnandiol-3(α),20-(α), was isolated. The final product melted at 182°; purification by chromatographic analysis did not raise the melting point. The dibenzoate (not previously described) melts at 167–168°. Since the dibenzoate of Compound B melts at 234–235°, it is clear that Compound B cannot be identical with pregnandiol-3(β),20(α). Compound B thus remains unidentified for the time being; it is probably an (*allo*) pregnane derivative containing a 3(β)-hydroxyl group.

TABLE I

COMPOUNDS (OTHER THAN CHOLESTEROL) ISOLATED FROM THE NEUTRAL, NON-SAPONIFIABLE FRACTION OF OX BILE

Compd.	M. p.,[12] °C.	Tentative empirical formula	Derivatives: Type	Derivatives: M. p.,[12] °C.
A	300	$C_{27}H_{40}O_3$	Monoacetate	216–217
			Monobenzoate	155–157
B	192–193	$C_{21}H_{36}O_2$	Dibenzoate	234–235
C	255–257	$C_{25-26}H_{40-42}O_4$	Monoacetate	187
D	232–233	$C_{24}H_{40}O_3$[13]	Monoacetate	111
E	202	$C_{24}H_{42}O_4$	Diacetate	142.5

Compound C contains either 25 or 26 carbon atoms. This is an unusual number for naturally occurring steroids but it might be mentioned that a C-26 acid has been obtained from bile.[6]

The ever-recurrent question of artifact formation deserves mention in this report but, since the

(1) Aided by a grant from G. D. Searle and Company.

(1a) Present Address: Department of Biology, Princeton University, Princeton, N. J.

(2) L. F. Fieser, "The Chemistry of Natural Compounds Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1937.

(3) K. Bloch, B. N. Berg and D. Rittenberg, *J. Biol. Chem.*, **149**, 511 (1943).

(4) H. Asikari, *J. Biochem.* (Japan), **29**, 319 (1939).

(5) W. Bergmann and Wm. T. Pace, THIS JOURNAL, **65**, 477 (1943).

(6) Y. Kurauti and T. Kazuno, *Z. physiol. Chem.*, **262**, 53 (1939).

(7) T. Kazuno, *ibid.*, **266**, 11 (1940).

(8) H. Makino, *ibid.*, **220**, 49 (1933).

(9) No attempt was made to isolate dihydrocholesterol which Schoenheimer, *et al.*,[10] have shown to be present to the extent of 1–2% in cholesterol from various organs; Pertzborn[11] reports 3.6% dihydrocholesterol in ox bile.

(10) R. Schoenheimer, H. V. Behring and R. Hummel, *ibid.*, **192**, 93 (1930).

(11) R. Pertzborn, *Z. ges. exptl. Med.*, **101**, 350 (1937).

(12) All m. p.'s in this paper are corrected.

(13) Empirical formula deduced from analytical data on acetate.

(14) A. Butenandt and G. Müller, *Ber.*, **71**, 191 (1938).

(15) R. E. Marker, O. Kamm, E. L. Wittle, T. S. Oakwood, E. J. Lawson and J. F. Laucius, THIS JOURNAL, **59**, 2291 (1937).