Mottern and Cole's method for the first time in obtaining the best results possible. Full acknowledgment is made to Mottern and Cole for their kindness in letting us use their method and for supplying us with goodly quantities of *d*-galacturonic acid.

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Sterols. LXXIV. Acetic Acid Derivatives of Estrone and α-Estradiol

BY RUSSELL E. MARKER AND EWALD ROHRMANN

Estratriene-1,3,5-one-17-oxyacetic acid-3 was first prepared by Ercoli and Mamoli[1] by treatment of estrone in aqueous potassium hydroxide solution with chloroacetic acid. In the present work the 3-oxyacetic acid derivatives of estratriene-1,3,5-one-17-ol-3 and of estratriene-1,3,5-diol-17(α),3 were prepared by the reaction of these substances with ethyl chloroacetate in the presence of an excess of an ethanolic solution of sodium ethylate. Estratriene-1,3,5-one-17-ol-3 was also caused to react in a similar way with α-chloropropionic acid to yield estratriene-1,3,5-one-17-oxymethylacetic acid-3. The acidic substances were characterized further by the formation of the methyl esters.

We wish to thank Parke, Davis and Company for their generous support and assistance rendered during the course of this work.

Experimental Part

Estratriene-1,3,5-one-17-oxyacetic Acid-3.—To a boiling solution of 1 g. of estrone in 40 cc. of absolute ethanol was added 3.5 cc. of ethyl chloroacetate and a solution of 600 mg. of sodium in 20 cc. of ethanol. The mixture was refluxed on the steam-bath for fourteen hours after which 2 g. of potassium hydroxide was added and the refluxing continued for one hour. The mixture was diluted with water and the clear solution acidified with hydrochloric acid. The white solid was taken up in ether and the ethereal solution washed with water and 5% sodium carbonate solution. Evaporation of the ether yielded approximately 150 mg. of unreacted estrone.

The sodium carbonate washing was acidified with hydrochloric acid and the white solid taken up in ether. Evaporation of the ether gave a product which crystallized from aqueous acetone as small white plates, m. p. 209–211°; yield, 750 mg.

Anal. Calcd. for $C_{20}H_{24}O_4$: C, 73.2; H, 7.4. Found: C, 73.5; H, 7.5.

When the reaction was carried out using equivalent amounts of sodium and ethyl chloroacetate, only a poor yield of the acid was obtained.

A solution of 50 mg. of the acid in 10 cc. of 80% ethanol was refluxed for one hour with 75 mg. of hydroxylamine hydrochloride and 100 mg. of sodium acetate. The product was crystallized from 80% ethanol to give white crystals of the **oxime**, m. p. 230–232° dec.

Anal. Calcd. for $C_{20}H_{25}O_4N$: C, 69.9; H, 7.3. Found: C, 70.0; H, 7.4.

The **methyl ester** was prepared by treating 150 mg. of the acid in ether–methanol solution with an excess of an ethereal solution of diazomethane. The product was crystallized from aqueous acetone as white crystals, m. p. 130–132°.

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.6; H, 7.7. Found: C, 73.5; H, 7.8.

Estratriene-1,3,5-ol-17(α)-oxyacetic Acid-3.—This was prepared from estratriene-1,3,5-diol-3,17(α) as described for estratriene-1,3,5-one-17-oxyacetic acid-3. The product was crystallized from aqueous acetone to give white crystals, m. p. 182–184°.

Anal. Calcd. for $C_{20}H_{26}O_4$: C, 72.7; H, 7.9. Found: C, 72.6; H, 7.9.

Treatment of the acid in ether–methanol solution with diazomethane yielded the **methyl ester** which crystallized as white crystals from ether–pentane, m. p. 94–96°.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.2; H, 8.2. Found: C, 73.3; H, 8.1.

Estratriene-1,3,5-one-17-oxymethylacetic Acid-3.—This was prepared from estrone and α-chloropropionic acid as described for the preparation of estratriene-1,3,5-one-17-oxyacetic acid-3 except that about three times as much sodium was used. The product was crystallized from aqueous acetone as white crystals, m. p. 195–198°.

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.6; H, 7.7. Found: C, 73.3; H, 7.7.

Treatment of the acid in methanol–ether solution with diazomethane yielded the **methyl ester** which crystallized from aqueous acetone as small white crystals, m. p. 137–139°.

Anal. Calcd. for $C_{22}H_{28}O_4$: C, 73.9; H, 7.9. Found: C, 74.1; H, 7.9.

SCHOOL OF CHEMISTRY AND PHYSICS
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(1) Ercoli and Mamoli, *Gazz. chim. ital.*, **68**, 142 (1938).

Identification of Propionic Acid in the Presence of Acetic and Butyric Acids

BY LOUIS MUSICANT AND FRANK J. KASZUBA

Pure propionic acid may be tested for readily in a number of ways, namely, as a derivative of *p*-toluidine,[1] benzylisothiourea[2] and the like; by the formation of 2-ethylbenzimidazole[3]; by its reac-

(1) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley & Sons, Inc., New York, N. Y., pp. 95, 144.
(2) S. Veibel and H. Lillelund, *Bull. soc. chim.*, [5] **5**, 1153 (1938).
(3) W. O. Pool, H. J. Harwood and A. W. Ralston, THIS JOURNAL, **59**, 178 (1937).

tion with lanthanum nitrate[4]; the behavior of its aqueous iron or copper salt solutions in the presence of amyl alcohol or other immiscible solvents,[5] etc. However, when acetates or butyrates, or both, are also present, some or all of the foregoing tests become inconsistent and unreliable. It is true such mixtures may be analyzed, both qualitatively and quantitatively, with exceptional success by the use of the methods of C. H. Werkman and co-workers.[6] Unfortunately, these procedures require very careful manipulation and are rather time consuming.

A need for a rapid and reliable means of identifying propionates in the presence of the above-mentioned interfering ions was satisfactorily filled by the use of mercurous nitrate.

Mercurous propionate crystallizes in a well-defined form which is quite different from either mercurous acetate or butyrate. Further, it forms readily even when the latter substances are also present. Once one has become familiar with the crystalline characteristics of the pure salts and their mixtures, the possibility of error becomes insignificant. The authors testing numerous unknowns, obtained results which were correct in each and every case.

The accompanying photomicrographs illustrate the crystal structures involved. The entire method is brief, simple, and does not require undue care or technique.

In the accompanying photomicrographs the concentrations of the salts were as follows: for Figs. 1, 2 and 3, 2% solutions of the pure sodium salts were used; for the remaining four, equal amounts of 2% solutions of the sodium salts of the ions involved were taken.

Experimental.—A neutral hydrolysate or test solution is vacuum distilled to dryness. The residue is then acidified with concentrated phosphoric acid and the liberated acids are distilled off *in vacuo*. It is advisable to collect the distillate in an 8″ × 1″ (20 × 2.5 cm.) test-tube surrounded by an efficient freezing mixture (salt, 33; pulverized ice, 100; −21°). The test-tube may be connected to the side-arm of the flask by means of a bent piece of ordinary glass tubing. This delivery tube should dip to within 2.5 cm. of the bottom of the receiver.

A capillary introduced into the semi-solid distillate (when acetate is present) will withdraw several drops of acid. This is transferred to a glass slide and a drop of 5% sodium carbonate solution is added (care being taken that the mixture still reacts acid to litmus) and the whole is evaporated to dryness. The residue is dissolved in 2–4 drops of water, and a drop of a saturated solution of mercurous nitrate (acidified with nitric acid to prevent decomposition) is placed alongside, and is allowed to flow into the former. The precipitate is examined immediately with a microscope.

To obtain the photomicrographs, the following procedure was used: one drop of a 2% aqueous solution of sodium propionate (or acetate, or butyrate, or mixtures of any two or all three) was placed on a glass slide and diluted with 2–4

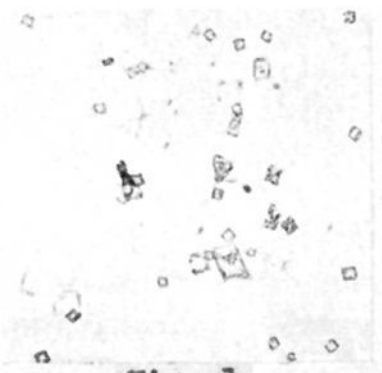

Fig. 1

Fig. 2

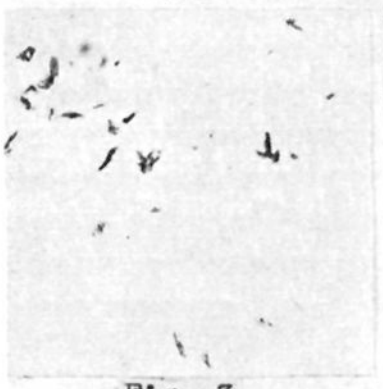

Fig. 3

Fig. 4

Fig. 5

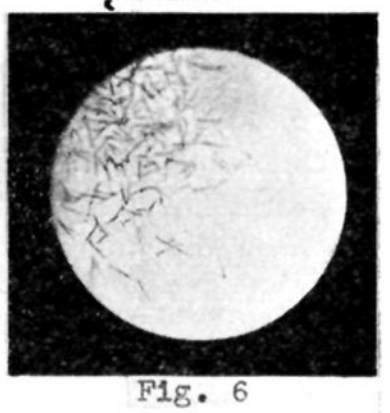

Fig. 6

Fig. 7

Mercurous salts: 1, acetate; 2, propionate; 3, butyrate; 4, acetate-propionate; 5, acetate-butyrate; 6, propionate-butyrate; 7, acetate-propionate-butyrate.

(4) A. Damour, *Compt. rend. acad. sci. Paris*, **43**, 976 (1857); *Chem. Zentr.*, **28**, 127 (1857).

(5) D. C. Dyer, *J. Biol. Chem.*, **28**, 445 (1917).

(6) O. L. Osburn, H. G. Wood and C. H. Werkman, *Ind. Eng. Chem., Anal. Ed.*, **8**, 270 (1936).

drops of water. A drop of the reagent was then allowed to flow into the test sample (magnification, 100 ×).

Discussion.—The distillation is employed to remove substances which may interfere with the analysis. It also serves to concentrate the acids present. Incidentally, too large an excess of acetic acid is conveniently and simultaneously frozen out.

If only the sodium salts of the acids involved comprise the test substance, the distillation may be dispensed with if one can be sure of at least 0.15 mg. of propionate in the test drop.

The method also may be used to identify either acetates or butyrates when they are present alone. As little as 0.15 mg. present in the test drop permits positive identification.

Formates tend to interfere with the analysis when the concentration is equal to or greater than either of the other anions. When smaller amounts are present, however, propionates may be identified although the crystal structure is slightly altered. Hence, if formates are indicated in a test solution (by the reduction of silver nitrate or mercuric chloride), it is advisable to destroy or otherwise remove them.

Attempts were made to employ other cations for the detection of the ions discussed under similar conditions. Thus, silver, bismuth, antimony, and lead salts of the acids were studied. Also, various cobaltammine complexes were tried. However, none of these were found to be completely suitable.

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Saponins and Sapogenins. XIV. The So-called Pyridazine Derivatives of Steroid Diones

BY C. R. NOLLER

The fact that the diketone obtained by the oxidation of chlorogenin[1] has been found to react with hydrazine to give a product the analysis of which checks that of a pyridazine derivative, has led Marker and Rohrmann[2] to postulate that the carbonyl groups of the diketone and hence the hydroxyl groups of chlorogenin occupy the 3,6-positions of the sterol nucleus. Several factors have led us to believe that this is not a likely structure for chlorogenin. Since tigogenin on oxidation gives gitogenic acid, one would expect chlorogenin, if it had the 3,6-formulation, to give digitogenic acid, which is not the case. Moreover, if oxidation opened ring I between C-3 and C-4, a β-keto acid would be formed or if ring I opened between C-2 and C-3 but the nuclear configuration were different, one would expect the ketodibasic acid to be isomerized readily by alkali since digitogenic acid readily is converted to digitoic acid, but the ketodibasic acid was recovered unchanged after alkaline saponification of the dimethyl ester. The opening of ring II between C-6 and C-7 on oxidation would not be expected since in the further oxidation of digitogenic acid ring II is opened between C-5 and C-6 to give a ketotribasic acid, oxydigitogenic acid. Finally the surface film of digitogenin appears to be unlike that of chlorogenin since the former contracts rapidly under pressure giving a solid condensed film which is partially collapsed[3] whereas chlorogenin gives a gaseous film which does not collapse until the pressure reaches 18 dynes per centimeter and which remains mobile even up to 32 dynes per centimeter.[4]

Because of the possibility of polymolecular condensation,[5] we have determined the molecular weight of the condensation product formed from hydrazine and the diketone from chlorogenin and have obtained values of 1410, 1980 and 2770 for three different preparations compared with a calculated value of 425 for a monomolecular pyridazine derivative. It is evident that the formation of condensation products with hydrazine does not prove that the hydroxyl groups of chlorogenin are in the 3,6-positions although the fact that the condensation products are polymolecular does not exclude these positions.

Since molecular weight determinations have not been reported for similar compounds from other steroid diones,[6] it seemed desirable to determine the molecular weight of a typical so-called pyridazine derivative such as that from choles-

(1) Noller, THIS JOURNAL, **59**, 1092 (1937).

(2) Marker and Rohrmann, *ibid.*, **61**, 946 (1939).

(3) Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936).

(4) Noller, THIS JOURNAL, **60**, 1629 (1938).

(5) Zimmerman and Lochte, *ibid.*, **60**, 2456 (1938).

(6) Windaus, *Ber.*, **39**, 2256 (1906); Fernholz, *Ann.*, **508**, 217 (1934); Windaus, Inhoffen and Reichel, *ibid.*, **510**, 259 (1934); Coffey, Heilbron and Spring, *J. Chem. Soc.*, 738 (1936).