with nitro paraffins. Further investigations on the formation of these carbonyl compounds are in progress.

The analyses reported were carried out by A. A. Sirotenko of this Department. The nitro paraffins used in this study were obtained through the courtesy of the Commercial Solvents Corporation.

COMMUNICATION NO. 198 FROM THE
DEPARTMENT OF ORGANIC CHEMISTRY
AND ENZYMOLOGY
FORDHAM UNIVERSITY R. T. GILSDORF
NEW YORK 58, N. Y. F. F. NORD

RECEIVED JULY 27, 1950

THE PREPARATION OF RADIOACTIVE PROGESTERONE AND DESOXYCORTICOSTERONE LABELED IN RING A[1]

Sir:

The usefulness of tagged progesterone (I) and DOCA (II) in the study of the metabolism of these hormones and of the etiology of certain diseases of mal-adaptation prompted their preparation from 3-keto-Δ^4-etiocholenic acid methyl ester (III). Side chain labeled I has previously been reported.[2] Fission[3] of III with ozone yields (55%) the corresponding open ring A keto-acid, *i. e.*-3,5-seco-5-oxo-17-carbomethoxy-etiocholan-3-oic acid (IV), which, as its methyl ester V, adds, under the usual Reformatsky conditions, the elements of methyl bromoacetate (VI). Simultaneously the intermediate products undergo dehydration and cyclization back to III. Respectively, with carboxyl and methylene labeled VI, the products are III-3-C^{14} and III-4-C^{14}. Both series of reactions have been conducted; that with the more readily available VI-1-C^{14} is reported herewith. From III-3-C^{14}, 21-diazoprogesterone-3-C^{14} (VII) was obtained in the usual manner,[4,5] and converted, as described, to both I-3-C^{14} and II-3-C^{14} with a specific activity of approximately 250,000 counts per milligram per minute. All counts were determined in the windowless flow counter operating at 40–50% efficiency, and are expressed below as disintegrations registered per minute per millimole.

V (formed with diazomethane from 3.17 g. of IV) was subjected to the Reformatsky reaction with 2 molar proportions of VI-1-C^{14} (2.8 g., containing approximately 3 millicuries of C^{14}).[6] The ether soluble neutral reaction products were refluxed (5 hours) in 20% concentrated hydrochloric acid in acetic acid, and then absorbed on alumina (90 g.). Fractional elution gave 300 mg. of III-3-C^{14}, melting at 130–132° (no depression on admixture with III) and counting 1.5×10^8. To the mother liquors of the above, 300 mg. of carrier III was added, and the mixture was recrystallized to yield a further 300 mg. of III-3-C^{14} counting at 7.3×10^6. The two were combined and saponified (4 hours reflux in 6% methanolic KOH) to the free etio acid (400 mg.). Its acid chloride, formed through the action of oxalyl chloride on the sodium salt,[5] was subjected to the usual Arndt–Eistert reaction[4,5] with diazomethane to give 240 mg. of 21-diazoprogesterone-3-C^{14} (VII), m. p. and mixture m. p. 163–170°.

Desoxycorticosterone-3-C^{14} 21-acetate (65 mg.) was obtained from VII (125 mg.) on hydrolysis with acetic acid.[4,5] The final product, separated and purified by sublimation, had m. p. and mixture m. p. 151–153°, count 8.4×10^7.

Progesterone-3-C^{14} was derived from VII through the general reaction between a diazoketone and HI described by Wolfrom and Brown.[7] VII (100 mg.), in chloroform, was shaken with concentrated hydriodic acid (liberation of iodine). Evaporation and crystallization yielded 70 mg. of I-3-C^{14}, m. p. and mixture m. p. 122–125°, count, 8.4×10^7.

Full experimental details will be reported later.

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[1] Aided by grants from the National Cancer Institute, U. S. Public Health Service, the Medical Research Division of the National Research Council (Ottawa), and Charles E. Frosst & Co., Montreal.

[2] Riegel and Prout, *J. Org. Chem.*, **13**, 933 (1948); MacPhillamy and Scholz, *J. Biol. Chem.*, **178**, 37 (1949).

[3] Reichstein and Fuchs, *Helv. chim. acta*, **23**, 676 (1940).

[4] Steiger and Reichstein, *ibid.*, **20**, 1164 (1937).

[5] Wilds and Shunk, THIS JOURNAL, **70**, 2427 (1948).

[6] Kindly prepared by Mr. B. Belleau.

[7] Wolfrom and Brown, THIS JOURNAL, **65**, 1516 (1943).

DEPARTMENT OF BIOCHEMISTRY
MCGILL UNIVERSITY R. D. H. HEARD
MONTREAL, CANADA P. ZIEGLER

RECEIVED JULY 17, 1950

THE CHEMICAL NATURE OF A UNIQUE FATTY ACID

Sir:

In connection with studies on the relation between biotin and fatty acids[1] we investigated the chemical nature of the fatty acids of *Lactobacillus arabinosus*. Distillation of the methyl esters, derived from the saponifiable fraction from 1.3 kg. of this organism, yielded, in addition to other materials, a fraction boiling at 187–188° at 3 mm. Saponification of this fraction gave an optically inactive, "branched chain" fatty acid (I), melting at 28–30°, of the composition $C_{19}H_{36}O_2$ (*Anal.* Calcd.: C, 76.96; H, 12.24; neut. eq., 296; C-methyl, 2. Found: C, 76.83; H, 12.54; N.E., 300; C-methyl, 1.3). The fatty acid exhibited an X-ray diffraction pattern indicating a chain length in the range of a C_{18} acid. The pattern was clearly distinguishable from those of known C_{18} fatty acids.[2] The compound failed to react with potassium permanganate in acetone and remained unchanged upon exposure to monoperphthalic acid. However, one mole of hydrogen was absorbed on catalytic hydrogenation

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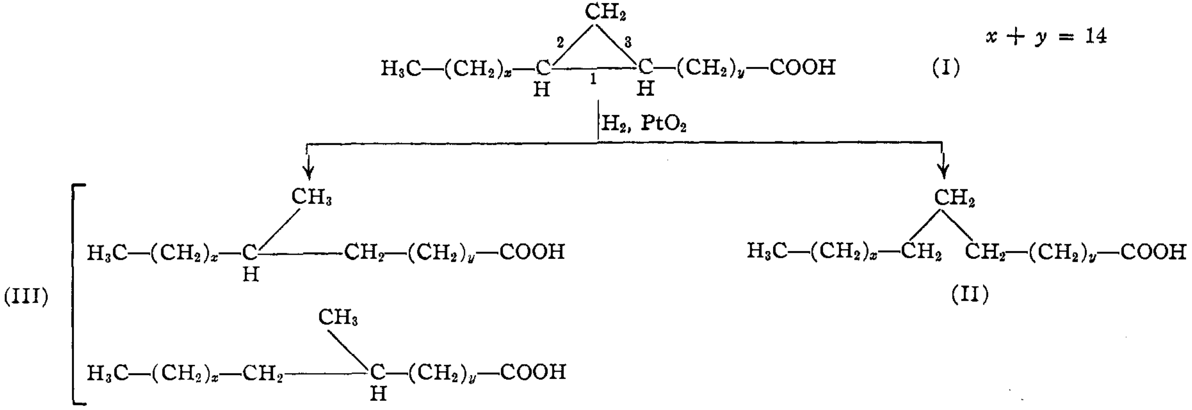
[1] Hofmann and Axelrod, *Arch. Biochem.*, **14**, 482 (1947).

[2] We are indebted to Dr. E. S. Lutton, The Procter & Gamble Company, for the X-ray work.

to give two new materials. One of these, a solid fatty acid melting at 68–68.5°, was, by mixed-melting point determinations and X-ray diffraction studies, unequivocally identified as nonadecanoic acid (II). The other melted at 13–14° and gave analytical figures in agreement with a composition of $C_{19}H_{38}O_2$ (*Anal.* Calcd.: C, 76.44; H, 12.83; neut. eq., 298; C-methyl, 2.0. Found: C, 76.29; H, 12.32; neut. eq., 301; C-methyl, 1.3) (tribromoanilide, m. p. 90–94°). The low melting point of this branched-chain, saturated C_{19} acid fraction suggests the presence of a mixture of methyloctadecanoic acids (III).[3]

The behavior of (I), *i. e.*, (1) its stability toward oxidation; (2) its lability toward hydrogenation, and (3) the formation of at least two hydrogenation products, one of which contains a straight carbon chain (nonadecanoic acid), points to the presence of a cyclopropane ring. The infrared absorption maximum at 9.8μ supports this conclusion[4]. As a working hypothesis we propose structure (I) for this fatty acid. Such a formula could well explain the experimental results presented. Thus its hydrogenation could yield the observed hydrogenation products through hydrogenolysis of bonds 1, 2 or 3 in the manner illustrated.



Liquid fraction, m. p. 13–14°

Solid acid m. p. 68–68.5°

Further work on the exact location of the cyclopropane ring as well as on the synthesis of acids of the general structure (I) is presently underway in this laboratory.

(3) Cason and Winans, *J. Org. Chem.*, **15**, 139 (1950).

(4) Derfer, Pickett and Boord, THIS JOURNAL, **71**, 2482 (1949).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF PITTSBURGH
PITTSBURGH, PENNSYLVANIA
KLAUS HOFMANN
ROBERT A. LUCAS

RECEIVED JULY 13, 1950

ELECTROMIGRATION ON FILTER PAPER[1]

Sir:

The recent publication of Cremer and Tiselius[2] of experiments on the electrophoresis of proteins in filter paper prompts us to report similar experiments on the electromigration of inorganic ions. Filter paper electromigration appears to be a rapid method for the determination of the sign of the charge of complex ions and for obtaining estimates of the stability constants of complexes.

The technique consists of moistening a strip of filter paper (E.D. No. 627-030; dimensions, 11 × 3 inches) with the supporting electrolyte, blotting off excess, placing a number of one microliter aliquots of solutions of the ions in question near the center of the paper and measuring the displacement (spot test developing) after applying a known d.c. voltage for a known period of time. The non-polarizable electrodes (silver strips for chloride solutions) are clamped to the paper, whose center portion is cut to *ca.* $^1/_3$ of the total width to increase the voltage drop in the vicinity of the test spots and to provide electrode compartments of fair capacity. For cooling the paper is placed on a 1″ insulated copper block. Temperature is measured with a small thermocouple. Evaporation is reduced by covering the paper with a glass plate to which narrow silver strips are cemented to permit measurement of the voltage drop in the vicinity of the spots. Depending on the conductivity of the solution voltage drops of 3–10 volts/cm. in the test region can be used without rise in temperature of more than 1–2° permitting completion of experiment in as little as 10 to 30 minutes. The extent of water movement primarily due to electro-endosmosis is estimated from the displacement of an "indicator" of zero charge. All data are corrected for adsorption on filter paper by determining the *R* values by standard paper chromatography.

Typical series of results at 26 ± 1° for mercuric ions and, for comparison of iodide ions, in chloride solutions are shown in Fig. 1. The experiments were carried out with hydrochloric acid except for *M* $Cl^- \leq 0.5$ where Cl^--ClO_4^- mixtures of ionic strength 0.5 containing 0.1 *M* H_3O^+ were used. While the mobility (sq. cm. $min.^{-1}$ $volt^{-1}$) of iodide ions remained practically constant and independent of chloride concentration that of

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[1] This document is based on work performed for the Atomic Energy Committee at the Oak Ridge National Laboratory.

[2] H. Cremer and A, Tiselius, *Biochem. Z.*, **320**, 273 (1950).