cis-**1,2-Dibenzoyl-1-*n*-propoxyethylene** was prepared from 2,5-diphenyl-3-*n*-propoxyfuran by the general method[9] described in the experiment just preceding. After several recrystallizations from ethanol and isopropyl alcohol the material melted at 72–73°; the yield was 77%.

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.17. Found: C, 77.18; H, 6.16.

The propoxyethylene was converted back to the furan by reduction with zinc and acetic acid.[6]

cis-**1,2-Dibenzoyl-1-isopropoxyethylene** was prepared from 2,5-diphenyl-3-isopropoxyfuran[5] in propionic acid at 0° by the general method[9] described above. The yield of material melting at 108–109° after recrystallization from isopropyl alcohol was 78%.

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.17. Found: C, 77.74; H, 6.44.

Zinc and acetic acid reduction[6] converted the material back to the furan from which it was made.

The difference in melting points between 1,2-dibenzoyl-1-*n*-propoxyethylene and 1,2-dibenzoyl-1-isopropoxyethylene makes them easily distinguished. Thus the corresponding furans can easily be distinguished through these derivatives. They are otherwise difficult to differentiate between, since the isopropoxyfuran melts at 87–88°, the *n*-propoxyfuran at 86–87° and the mixture melting point between the two is 82–85°.

Acknowledgments.—The authors wish to thank the Research Corporation, New York, N. Y., for a grant-in-aid which helped make this work possible. They also wish to thank Mr. Elias E. Kawas for carrying out three of the alcoholysis experiments.

Summary

1. 3-Alkoxy-2,5-diphenylfurans have been found to undergo alcoholysis, termed transetherification, under acidic conditions.

2. A comparison is made of transetherification, transesterification and the addition of alcohols to alkyl vinyl ethers.

3. The nitric acid oxidation of 2,5-diarylfurans to *cis*-1,2-diaroylethylenes has been extended to 3-alkoxy-2,5-diphenylfurans.

AUSTIN, TEXAS RECEIVED APRIL 25, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

A Glyco-lipide Produced by *Pseudomonas Aeruginosa*[1]

BY F. G. JARVIS[2] AND M. J. JOHNSON

Several crystalline compounds[3,4,5,6,7] and at least two partially purified oils[8,9] have been isolated from *Pseudomonas aeruginosa* cultures. These materials have been primarily investigated with respect to their antibacterial activity. With the exception of pyocyanin,[3,4] none of these metabolic products have been fully characterized. The isolation and investigation of the structure of an acidic, crystalline glyco-lipide produced by *P. aeruginosa* is described in this paper. The compound contains the same structural units (L-rhamnose and *l*-β-hydroxydecanoic acid) as the oil isolated by Bergström, *et al.*,[9] but has a higher molecular weight and a higher rhamnose–hydroxy-acid ratio. It is quite possible that the compound is identical to the crystalline material isolated by Birch-Hirschfeld[6] but insufficient data are available for suitable comparison. Our compound was found to be bacteriostatic to *Mycobacterium tuberculosis* H37 Rv in a concentration of about 0.5 mg. per ml. of culture medium. Five mg. given introperitoneally to mice killed in about sixteen hours.[10]

Isolation.—The organism was grown on a 4% Difco peptone–3% glycerol broth at 30° on a reciprocating shaker. Cultures were harvested after from four to five days growth. The acid was produced by each of three strains of *P. aeruginosa* tested (University of Washington strains no. 141, 142, 261). The one strain of *P. florescens* tested failed to produce the compound. Yields as high as 2.5 g. per liter were obtained. The crystalline acid was not produced (or could not be isolated by our procedure) when the peptone was replaced by tryptone or the glycerol replaced by glucose in the growth medium.

The compound was obtained in crystalline form by acidifying the whole culture to *p*H 2 with sulfuric acid and refrigerating for two or three days. The crystals were collected on very coarse filter paper, taken up in a small volume of ethyl ether (insoluble residues discarded) and precipitated by addition of petroleum ether. After the removal of solvent by decantation and evaporation, the precipitate was dissolved in dioxane and crystallized by adding water and chilling. The material could be recrystallized in the cold by acidifying water solutions of its sodium salt or by adding water to dioxane or acetone solutions of the free acid.

Properties.—The compound crystallizes in the form of thin, colorless rectangular platelets. It is very soluble in ether, ethyl alcohol, acetone, dioxane and dilute sodium bicarbonate solution. It is nearly insoluble in water and petroleum ether. The compound (m. p. 86°, α_D = −84°, 3% in chloroform) has a neutral equivalent of about 665. Molecular weight determinations by the Rast method gave a value of about 650.

Anal.[11] Calcd. for $C_{32}H_{58}O_{14}$: C, 57.64; H, 8.77. Found: C, 57.62; H, 8.74.

Other data (see below) indicate that the true formula is $C_{32}H_{60}O_{14}$. Quantitative acetylation of the material showed 4.3 hydroxyl groups per mole; however, chromatographic analysis of the acetylated material gave three acidic bands indicating either incomplete acetylation or decomposition during the reaction. The chromatographic

(1) Published with the approval of the Director of the Wisconsin Experiment Station.

(2) National Institute of Health Predoctorate Research Fellow.

(3) F. Wrede and E. Strack, *Ber.*, **62B**, 2051 (1929).

(4) H. Hillemann, *ibid.*, **71B**, 46 (1938).

(5) S. Hosoya, *Compt. rend. soc. biol.*, **99**, 771 (1928).

(6) L. Birch-Hirschfeld, *Z. Hyg. Infektionsrank.*, **116**, 304 (1935).

(7) E. E. Hays, *et al.*, *J. Biol. Chem.*, **159**, 725 (1945).

(8) R. Schoental, *Brit. J. Exp. Path.*, **22**, 137 (1941).

(9) S. Bergström, H. Theorell and H. Davide, *Arkiv Kemi Mineral. Geol.*, **23A**, No. 13 (1947).

(10) The authors are indebted to Dr. Russell S. Weiser, University of Washington, for the antibiotic and mouse toxicity tests.

(11) The carbon–hydrogen analyses were performed by the Clarke Microanalytical Laboratory, Urbana, Illinois.

procedure of Ramsey and Patterson[12] was employed, modified to the extent that Celite 545 was used as the supporting phase in place of silica gel. Tests for other active groups were negative. The compound undergoes acid hydrolysis. Periodate oxidation resulted in the uptake of 2 atoms of oxygen per mole of compound. The compound gives a positive Molisch test but does not reduce Fehling solution. A loss of weight equivalent to 1 water molecule per mole of acid occurs upon heating under reduced pressure to 60° for twenty-four hours. The weight loss is more rapid at higher temperatures. The resultant product (m. p. 60–64°) could not be crystallized from dry solvents. When crystallized from aqueous solutions, the original acid (m. p. 86°) was obtained.

Degradation.—The water-soluble fraction obtained by acid hydrolysis (1 *N* hydrochloric acid in 50% redistilled dioxane for two hours at reflux temperature) was obtained in pure crystalline form by neutralization to *p*H 7, removal of solvent by distillation under reduced pressure, extraction of the residue with absolute ethyl alcohol, and crystallization from acetone–ethanol or acetone–water mixtures. The crystalline material gave the usual qualitative tests for an aldose-type monosaccharide.

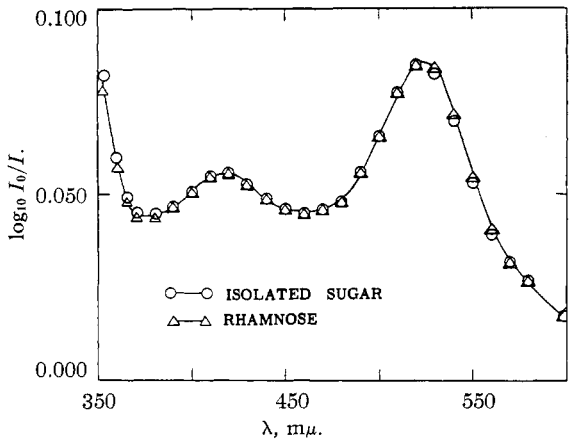


Fig. 1.—Absorption spectra of carbazole–sugar reaction mixtures. Sugar concentrations were 15γ/ml. of reaction mixture: O—O, isolated sugar; △—△, rhamnose.

The absorption spectrum of its carbazole reaction product in sulfuric acid[13,14,15] was determined (Beckman photometer model A, 1-cm. Corex cell and tungsten filament lamp), and found to be identical with that for L-rhamnose (Fig. 1). Although the efficacy of this method has been questioned,[15] we have found that the differences in the absorption spectra (340–600 mμ) of the sugars tested (glucose, galactose, mannose, arabinose, xylose and rhamnose) are more than sufficient for the qualitative indentification of pure sugars if suitably controlled conditions are employed. The sugar (α_D = +8.4°, 5% solution in water) crystallized in two forms, the usual hydrated form (m. p. 92–93°) and the anhydrous form (m. p. 121–123°). A phenylosazone was also prepared (m. p. 179–180°).

Anal. (hydrated form). Calcd. for $C_6H_{14}O_6$: C, 39.56; H, 7.75. Found: C, 39.75; H, 7.69.

All of these data indicate that the sugar is L-rhamnose.

Two moles of reducing sugar were shown to be present in each mole of original acid by quantitative measurement of of hydrolysis (Fig. 2). Quantitative sugar analyses were made by the Shaffer and Somogyi method[16] with their reagent 50 with 5 g. of potassium iodide. Titrations were referred to a standard rhamnose curve. The rates of release of the two reducing groups were quite different, the first being released much more readily than the second. When hydrolysis was halted after the release of the first mole of sugar and the reaction mixture extracted with petroleum ether, the second mole of sugar (unhydrolyzed) was found in the petroleum ether fraction. The sugar present in each fraction was found to be L-rhamnose after isolation.

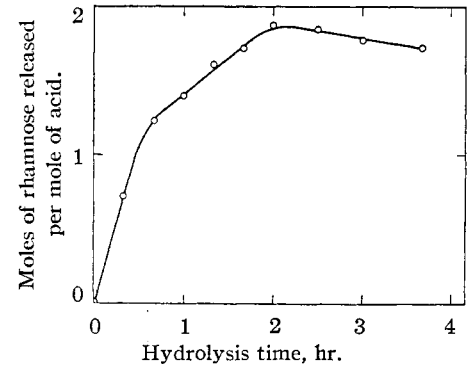


Fig. 2.—Acid hydrolysis of isolated crystalline acid: the reaction mixture contained 200 mg. of isolated acid in 50 ml. of 1 *N* hydrochloric acid in 50% redistilled dioxane at zero hours. Hydrolysis was conducted at reflux temperature and 1 atm.

No other water-soluble compound could be found in appreciable quantity after complete hydrolysis.

The ether-soluble fraction from a two-hour acid hydrolysis was examined by chromatography.[12] Two major acidic fractions were isolated, one of which passed through the column very rapidly and the other much more slowly. The slow component, after separation from small amounts of two closely related impurities by chromatography, was recrystallized from petroleum ether.

Anal. Calcd. for $C_{10}H_{20}O_3$: C, 63.80; H, 10.71. Found: C, 63.93; H, 10.52.

The acid was shown to contain one hydroxyl group per mole by quantitative acetylation followed by chromatography. Two acids were recovered from the column, one with a neutral equivalent of 232 (acetylated acid) and one with a neutral equivalent of 170 (probably dehydrated acid). Titration of the acetylated material produced corresponded closely to theory for the amount of acetic anhydride used. The isolated acid (α_D = −21°, 2.5% solution in chloroform) melted at 47–48°. The S-benzylthiouronium salt melted at 129–130°. Upon oxidation by chromic acid by the method used by Bergström, *et al.*,[9] a volatile, optically inactive liquid acid (neut. equiv. 145) was obtained which was chromatographically identical to *n*-caprylic acid. The chromatographic method employed[12] was found to be sufficiently efficient to positively differentiate between *n*-pelargonic, *n*-caprylic and *n*-enanthic acids. The amide of the oxidized acid was prepared (m. p. 105–106°). A mixed melting point with *n*-caprylamide showed no depression.

Anal. Calcd. for $C_8H_{17}ON$: N, 9.78. Found (Kjeldahl): N, 9.85.

These data indicate that the isolated acid is normal *l*-β-hydroxydecanoic acid although the possibility of its being a branched chain isomer has not been rigorously excluded.

The other major component of the ether-sol-

(12) L. L. Ramsey and W. I. Patterson, *J. Assn. Official Agr. Chem.*, **31**, 139 (1948).

(13) S. Gurin and D. B. Hood, *J. Biol. Chem.*, **131**, 211 (1939).

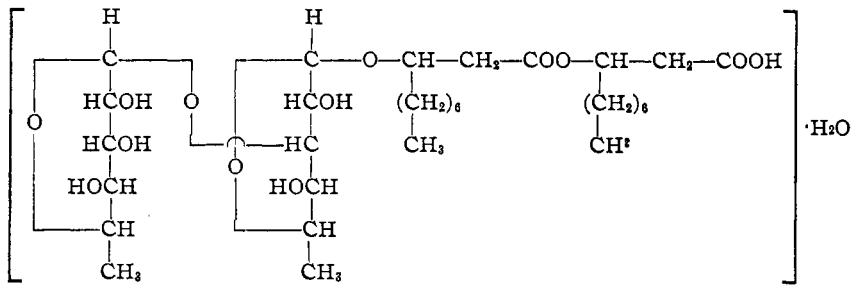
(14) S. Gurin and D. B. Hood, *ibid.*, **139**, 775 (1941).

(15) G. Holzman, R. V. MacAllister and C. Niemann, *ibid.*, **171**, 27 (1947).

(16) P. A. Shaffer and M. Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).

uble hydrolytic fraction could not be obtained in crystalline form. It proved to have a neutral equivalent of about 360 and yielded two moles of β-hydroxydecanoic acid upon saponification. As no such ester is formed by the conditions employed during hydrolysis, and as the proportion of this compound decreases with hydrolysis time, it was concluded that two moles of *l*-β-hydroxydecanoic acid exist in esterified form in the original acidic compound.

The following formula is presented as being most compatible with the accumulated data. The 1,3-linkage shown is, of course, largely speculative as the only pertinent data obtained were the uptake of only two atoms of oxygen per mole of compound by periodate oxidation. Assuming a pyranoside-type ring, either the 1,2- or 1,4-linkages would result in a structure which would normally take up three oxygen atoms. No direct evidence for the type of sugar linkages involved (α or β) was obtained.



Summary

A crystalline, acidic glyco-lipide was produced by three strains of *Pseudomonas aeruginosa* on peptone–glycerol broth.

This compound was found to contain two units each of L-rhamnose and normal *l*-β-hydroxydecanoic acid.

The most likely formula on the basis of the available data is proposed.

MADISON 6, WIS. RECEIVED JULY 18, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, JEFFERSON MEDICAL COLLEGE]

The Preparation of 3,4,5-d_3-Lithocholic Acid[1]

BY W. H. PEARLMAN, M. R. J. PEARLMAN AND S. ELSEY

Schoenheimer and Berliner[2] described a procedure whereby deuteriolithocholic acid might be obtained by reduction of methyl $\Delta^{4,5}$-3-ketocholenate in ethanol–ether solution in the presence of platinum oxide; the isotopically labelled bile acid should prove useful for metabolic study. However, their work was carried out with hydrogen and not with deuterium. On repeating their procedure but using deuterium, we obtained lithocholic acid containing only 0.35 atom % excess deuterium whereas the theoretical value for 3,4,5-d_3-lithocholic acid is 7.50. This result is not altogether surprising in view of the observation by Anderson and MacNaughton[3] that deuterium is exchanged for the hydroxyl hydrogen of alcohols such as isopropyl alcohol in the presence of catalysts; evidence was obtained, however, that exchange did not occur with isopropyl alcohol as long as any acetone was present. When absolute ether was substituted for ethanol–ether in the catalytic reduction, lithocholic acid was obtained containing 1.92 atom % excess deuterium. Further investigation of optimal conditions for catalytic deuteration of methyl $\Delta^{4,5}$-3-ketocholenate appeared to be desirable; the results of such an investigation are summarized in Table I. The most satisfactory procedure found was the following. Reduction of methyl $\Delta^{4,5}$-3-ketocholenate in ether using a 5% palladium on charcoal catalyst gave a mixture consisting principally of methyl 3-ketocholanate and some methyl 3-ketoallocholanate; the mixture contained 3.59 atom % excess deuterium. Deuteration of this mixture in ether in the presence of platinum oxide yielded lithocholic acid with a slightly higher value, 3.72 atom % excess deuterium. Another run was made under identical conditions starting with methyl $\Delta^{4,5}$-3-ketocholenate; lithocholic acid was obtained containing 3.89 atom % excess deuterium. Thus, it appears that two step deuteration of methyl $\Delta^{4,5}$-3-ketocholenate furnishes lithocholic acid with twice as much deuterium as that obtained in the single step procedure; yet, theoretically three deuterium atoms should enter the steroid molecule in either procedure.[4] By way of comparison, it is interesting that Schoenheimer,

(1) This investigation was supported by a grant-in-aid from the United States Public Health Service, under the National Cancer Institute Act.

(2) R. Schoenheimer and F. Berliner, *J. Biol. Chem.*, **115**, 19 (1936).

(3) L. C. Anderson and N. W. MacNaughton, THIS JOURNAL, **64**, 1456 (1942).

(4) Since there are 40 hydrogen atoms per molecule, the value corresponding to a content of three deuterium atoms is 7.5 atom% excess deuterium. A fourth deuterium atom is introduced but, being an hydroxyl deuterium, it is readily exchanged in subsequent manipulation.