The anhydrous hydrochloride melted at 295–297°.

Anagyrine perchlorate was prepared according to Ing as long needles soluble in hot water and crystallizing readily from cold, decomposing without melting at a high temperature.

Anal. Calcd. for $C_{15}H_{20}ON_2 \cdot HClO_4$ (344.7): C, 52.22; H, 6.15; N, 8.12. Found[9]: C, 52.13, 52.30; H, 6.37, 6.31; N, 8.10, 8.13.

Anagyrine Gold Chloride.—An aqueous solution of the hydrochloride was treated with excess of gold chloride and the resulting precipitate was recrystallized from boiling dilute hydrochloric acid after filtering from the gold precipitated by reduction. The double salt crystallized in long golden needles melting at 167–168° (uncor.) after desiccator-drying. Analysis indicated somewhat more than the theoretical gold content, possibly due to reduced gold.

Anal. Calcd. for $C_{15}H_{20}ON_2 \cdot HAuCl_4$ (584.2): Au, 33.75. Found: Au, 34.04, 34.09.

Anagyrine Picrate.—The hydrochloride in water treated with alcoholic picric acid gave a bright yellow crystalline precipitate which was collected and dried. It melted at 169.5° and did not depress the melting point of authentic anagyrine picrate. Attempts to recrystallize these picrates from water resulted only in crystals of picric acid.

Anagyrine Base.—Ten grams of the hydrochloride was alkalized with sodium hydroxide and the free alkaloid shaken out with ether. After removal of the solvent the base was distilled under reduced pressure. At 12 mm. the major portion distilled between 260 and 270°. The distillate was a light yellow oil that solidified to a yellow glass. In water solution it gave a red color with ferric chloride and in acid solution readily reduced potassium permanganate. It was levorotatory in alcohol, $[\alpha]^{25}D$ −168°, l = 1, c = 1.0596, a = 1.78°.

Anagyrine Methiodide.—The base, 2 g., in 15 cc. of acetone and 2 cc. of methyl iodide evolved heat and began to deposit crystals within thirty minutes. The crystals were recrystallized from methanol and dried at 110°. They melted at 262–263°.

Anal. Calcd. for $C_{15}H_{20}ON_2 \cdot CH_3I$: I, 32.86. Found: I, 32.77.

Other Bases.—The mother liquors from the hydrochloride were alkalized with sodium hydroxide in water and the liberated bases were shaken out with chloroform. A portion of the residue was shown to be anagyrine. No other base could be identified in the remainder. Cytisine, methylcytisine and sparteine were proved absent by appropriate tests.

Summary

Dry *Lupinus laxiflorus* var. *silvicola* C. P. Smith contains 0.7 to 1% of alkaloids, principally anagyrine. Cytisine, methylcytisine, and sparteine were not found in the plant. Anagyrine gives a characteristic blue color in the modified Grant test for sparteine.

WASHINGTON, D. C. RECEIVED SEPTEMBER 22, 1939

(9) The analyses were made by the Arlington Laboratories, V. A. Conard, Director.

[CONTRIBUTION NO. 389 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

A Method for the Preparation of α,β-Diglycerides of Fatty Acids

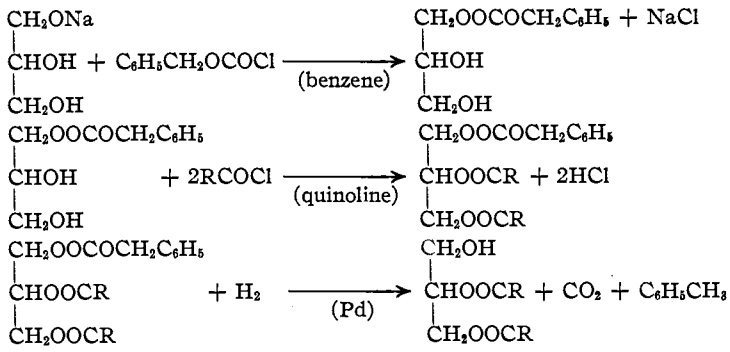
BY B. F. DAUBERT AND C. G. KING

The preparation and identification of α,β-diglycerides of the fatty acids has been difficult, due principally to the migration of acyl groups from the β- to the α′-position, as shown by Fischer.[1] Fairbourne and associates[2,3,4] have shown that the unsymmetrical diglycerides reported by earlier investigators[5,6,7,8,9] were probably the symmetrical isomers or mixtures in every case. The methods reported by Humnicki and Lunkiewicz,[10] Weizman and Haskelberg[11] and Delaby and Dubois[12] also involved reaction conditions that would almost certainly yield products containing little if any of the α,β-diglycerides.

The methods of Abderhalden and Eichwald[13] and Bergmann and associates,[14] using propylamine, and the trityl ether method of Helferich and Sieber,[15] have proved to be satisfactory for the preparation of aromatic α,β-diglycerides, but in no case have α,β-diglycerides of the fatty acids been prepared and characterized as pure compounds. Recently Verkade and associates[16] have outlined a general method for the preparation of α,β-diglycerides that should be satisfactory, involving the catalytic detritylation of α,β-diacyl-α′-trityl glycerol, but their work has not been reported in detail.

(1) Fischer, *Be.*, **53**, 1621 (1920).
(2) Fairbourne and Cowdrey, *J. Chem. Soc.*, 129 (1929).
(3) Fairbourne, *ibid.*, 389, 372 (1930).
(4) Fairbourne, Gibson and Stephens, *ibid.*, 445 (1931).
(5) Guth, *Z. Biol.*, **44**, 78 (1903).
(6) Grun and Theimer, *Ber.*, **40**, 1792 (1907).
(7) Renshaw, THIS JOURNAL, **36**, 537 (1914).
(8) Thomson, *Trans. Roy. Soc. Canada*, **20**, 445 (1926).
(9) Heiduschka and Schuster, *J. prakt. Chem.*, **120**, 145 (1928).
(10) Humnicki and Lunkiewicz, *Bull. soc. chim.*, **40**, 422 (1929).
(11) Weizman and Haskelberg, *Compt. rend.*, **189**, 104 (1929).
(12) Delaby and Dubois, *ibid.*, **187**, 767, 949 (1928).
(13) Abderhalden and Eichwald, *Ber.*, **49**, 2095 (1916).
(14) Bergmann, *et al.*, *ibid.*, **54**, 936 (1921); *Z. physiol. Chem.*, **137**, 27, 47 (1924).
(15) Helferich and Sieber, *ibid.*, **170**, 31 (1927); **175**, 311 (1928).
(16) Verkade, van der Lee, de Quant and Zuydewijn, *Proc. Acad. Sci. Amsterdam*, **40**, 580 (1937).

In the present investigation a new method of synthesis has been established, based upon the reaction between α-monosodium glyceroxide and benzyl chloroformate (carbobenzyloxy chloride), followed by esterification and catalytic reduction, as indicated in the following series of reactions



A brief study was also made of the solubilities and relative migration tendencies of aromatic and aliphatic unsymmetrical diglycerides.

Experimental

Preparation of 3-Carbobenzyloxyglycerol.—3-Monosodium glyceroxide was prepared by the method of Fairbourne and Toms.[17] To 5 g. of the oxide dissolved in 75 ml. of dry benzene there was added, in divided portions, 7.5 g. of benzyl chloroformate, previously prepared by the method of Bergmann and Zervas.[18] During the addition of the benzyl chloroformate the reaction mixture became warm. It was then allowed to stand twenty-four hours at room temperature, heated gently, filtered and the precipitate identified as sodium chloride. The filtrate was boneblacked, filtered and evaporated under reduced pressure. The residue consisted of a pale-yellow, oily liquid. Attempts to crystallize it were not successful. The yield was 8.2 g. (83%).

Anal. Calcd. for $C_{11}H_{14}O_5$: C, 58.40; H, 6.24. Found: C, 58.21, 58.16; H, 6.09, 6.16.

Preparation of 3-Carbobenzyloxy-1,2-dipalmitin.—To 5 g. of 3-carbobenzyloxyglycerol dissolved in 20 ml. of benzene and 5 ml. of quinoline there was added slowly a mixture of 5 g. of palmityl chloride and 5 ml. of quinoline. The mixture was shaken continuously for two hours and set aside at room temperature for four days. The product was then taken up in ether and the solution washed successively with cold 0.5 *N* H_2SO_4, saturated sodium bicarbonate, water and finally dried over anhydrous sodium sulfate. After evaporation *in vacuo* to dryness, the residue was redissolved in ether. Small white crystals separated after twenty-four hours at 5°, m. p. after recrystallization, 71°, yield 5 g. (80%).

Anal. Calcd. for $C_{43}H_{74}O_7$: C, 73.46; H, 10.61. Found: C, 73.09, 73.22; H, 10.73, 10.52.

Preparation of 1,2-Dipalmitin.—3-Carbobenzyloxy-1,2-dipalmitin (2 g.) was suspended in 75 ml. of absolute alcohol and transferred to a hydrogenation bottle together with 0.5 g. of palladium black. Reduction was complete after approximately two hours at room temperature and 30 lb. (2 atm.) pressure. The solution was filtered twice to remove the catalyst, then concentrated by evaporation and cooled for twenty-four hours. The 1,2-dipalmitin separated as flaky, white crystals, m. p. 64° after being recrystallized several times from absolute alcohol, yield 1.2 g. (76%).

Anal. Calcd. for $C_{35}H_{68}O_5$: C, 73.80; H, 12.05. Found: C, 73.89, 73.95; H, 11.67, 11.88.

Golendeev[19] reported a m. p. of 70–73° for 1,2-dipalmitin, but his method of esterifying the corresponding allyl halide (heating with the potassium salt of the fatty acid) would almost certainly cause a rearrangement of the structure to produce the 1,3-dipalmitin, m. p. 69.5°.

A second preparation of 1,2-dipalmitin, identical with the above product, was made by the method proposed by Verkade.[16] It was necessary to carry out the reduction at 45 lb. (3 atm.) pressure, 45–50°. For additional identification the triglyceride, 1-*p*-bromobenzoyl-2,3-dipalmitin, m. p. 68.8°, previously made by an independent method[20] was prepared from 1,2-dipalmitin by direct esterification.

Using the same procedure as outlined above for dipalmitin, 1,2-dimyristin was prepared, m. p. 59°, yield 80%.

Anal. Calcd. for $C_{31}H_{60}O_5$: C, 72.59; H, 11.79. Found: C, 72.25, 72.31; H, 11.56, 11.62.

The m. p. of the intermediate carbobenzyloxy-1,2-dimyristin was 67–68°.

Anal. Calcd. for $C_{39}H_{66}O_7$: C, 72.39; H, 10.28. Found: C, 72.11, 72.20; H, 10.06, 10.13.

Preparation of 3-Carbobenzyloxy-1,2-glyceroldibenzoate.—Essentially the same method as outlined above for 3-carbobenzyloxy-1,2-dipalmitin was followed for the preparation of the analogous dibenzoate.

Anal. Calcd. for $C_{25}H_{22}O_7$: C, 69.11; H, 5.10. Found: C, 68.86, 68.92; H, 5.17, 5.21.

Preparation of 1,2-Dibenzoate of Glycerol.—The 1,2-dibenzoate was prepared from the carbonate as described for 1,2-dipalmitin, but considerable difficulty was encountered in crystallizing the sirupy liquid remaining after evaporation of the solvent. Crystallization was accomplished after the product had been held two weeks in a vacuum desiccator, m. p. 58°, yield 70% (Helferich and Sieber[15] 58"–59°).

The 1,2-dibenzoate of glycerol thus obtained was used to prepare 1,2-dibenzoyl-3-trityl glycerol, m. p. 91°.

For further identification, the 1-*p*-bromobenzoyl-2,3-dibenzoate of glycerol, m. p. 107°, was prepared from the above 1,2-dibenzoate and also from 1-*p*-bromobenzoyl glycerol and benzoyl chloride.

Anal. Calcd. for $C_{24}H_{19}O_6Br$: Br, 16.54. Found: Br, 16.38, 16.45.

Migration of Acyl Groups.—When 1,2-dipalmitin was allowed to stand for twenty-four hours in solution in 0.1,

(17) Fairbourne and Toms, *J. Chem. Soc.*, **119**, 1035 (1921).
(18) Bergmann and Zervas, *Ber.*, **65**, 1194 (1932).
(19) Golendeev, *J. Gen. Chem. U. S. S. R.*, **6**, 1841 (1936).
(20) Daubert and King, THIS JOURNAL, **60**, 3003 (1938).

0.05 and 0.025 *N* alcoholic hydrochloric acid or ammonia (prepared by passing the gases into alcohol), there was a marked drop in melting point of the recovered product. There was no change in the melting point of 1,2-dibenzoyl glycerol, however, when identical tests were made, giving further evidence of the greater stability of the β-type aromatic esters compared to the β-glycerides of fatty acids.

Solubilities.—In an earlier publication[19] it was noted that the solubility ratios of the α- and β-aromatic and aliphatic monoglycerides in ether were reversed, the aromatic α-esters being more soluble than the β-isomers, but in the aliphatic series, the α-esters were less soluble than the β-isomers. A similar reversal of solubility in alcohol will be noted from the data in Table I.

TABLE I

SOLUBILITIES

Compound	Solvent	Temp., °C. (±0.01°)	Soly. g. per 100 ml.
α-Monopalmitin	Alcohol	25	4.09
β-Monopalmitin	Alcohol	25	4.61
α-*p*-Bromobenzoate	Alcohol	26	16.05[a]
β-*p*-Bromobenzoate	Alcohol	26	4.41[a]
β-*p*-Bromobenzoyl-α,α′-benzylidene glycerol	Alcohol	26	0.50
	Ether	26	1.15
β-Palmityl-α,α′-benzylidene glycerol	Alcohol	26	1.75
	Ether	26	17.40

[a] Repeated from an earlier paper for comparison.[20]

Summary

A new method for the synthesis of α,β- or 1,2-diglycerides has been described, in which sodium glyceroxide and benzyl chloroformate served as intermediates. Good yields were obtained and the reaction conditions made it possible to avoid the common β- to α-shift in structure.

1,2-Dipalmitin, m. p. 64°, 1,2-dimyristin, m. p. 59° and 1,2-dibenzoate of glycerol, m. p. 59°, were prepared and their structures were verified by making derivatives of known constitution.

A solution of 1,2-dipalmitin in 0.1 to 0.025 *N* alcoholic hydrochloric acid and ammonia underwent a rapid change in structure, but the analogous 1,2-dibenzoate was stable under the same conditions of exposure.

PITTSBURGH, PENNA. RECEIVED AUGUST 4, 1939

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

On the Effect of Acids in the General Meaning of the Term on the Activity of Invertase

BY WALTER ABBOTT WISANSKY

That the value of the *p*H of a solution in which an enzyme is acting, is a most important factor in controlling the activity of the enzyme, is well known. For example, the enzyme invertase has its maximum power for inverting sucrose in a range of *p*H values surrounding *p*H 4.5. If the *p*H value is raised or lowered sufficiently away from *p*H 4.5, the activity of invertase diminishes.

The explanation of the *p*H effect on enzymatic activity has been sought for a long time. The ionization hypothesis of Michaelis[1] has been a much favored suggestion in spite of certain shortcomings.[2] On the basis of this hypothesis invertase is supposed to be active only in the un-ionized condition.

In considering the effect of *p*H changes, workers have only been concerned with the effect produced on enzymatic activity by changes in the thermodynamic activity of one acid, oxonium ion. Since Brönsted[3] and others have demonstrated the generality of the term "acid," it became of interest to determine whether or not any acid, in the general meaning of the term, could alter the activity of enzymes. It was thought that if neutral acids could exert some effect on the activity of the enzyme invertase under conditions which purported to keep the degree of ionization of the enzyme constant (conditions such as constant ionic strength, and constant *p*H value), then one might impute to oxonium ion a role similar to that played by neutral acids in controlling the enzymatic activity of invertase, beyond oxonium ion's assured role of controlling the degree of ionization of the protein enzyme.

Since in practically all enzyme work, buffers are employed to regulate the *p*H, and since buffer solutions contain acids in the general meaning of the term, here is a very convenient source of acids whose effects on the activity of invertase it is proposed to consider.

The effects of neutral buffer acids on the activity of invertase have been studied.

In brief, the plan pursued in studying the effects of buffer acids on the activity of invertase

(1) L. Michaelis and H. Davidsohn, *Biochem. Z.*, **35**, 386 (1911).
(2) J. M. Nelson, *Chem. Rev.*, **12**, 1 (1933).
(3) J. N. Brönsted, *ibid.*, **5**, 231 (1928).