Acetylation produced **oleanolic acid acetate**, m.p. 262–265°, $[\alpha]^{27}D$ +77.6°; a mixture melting point with authentic material[3] was undepressed.

The original aqueous, alkaline filtrate was acidified, extracted with ether and the ether extract together with the mother liquors from the recrystallization of oleanolic acid was methylated with diazomethane. Chromatography of the crude methyl esters (3.0 g.) on 300 g. of alumina (deactivated with 12 cc. of 10% acetic acid) and elution with benzene led to a series of crystalline eluates. The first two fractions (200 cc.) were combined and recrystallized from methanol to furnish 0.34 g. of long needles of **methyl betulinate**, still slightly contaminated with methyl oleanolate, m.p. 214–222°, undepressed upon admixture with an authentic specimen, $[\alpha]^{28}D$ +3.8°; identity was further confirmed by the preparation of **acetyl methyl betulinate**, m.p 201–203°, $[\alpha]^{27}D$ +16°, and by direct infrared comparison in each case with authentic samples of the corresponding betulinic acid derivatives. These physical constants are in reasonably good agreement with the literature values,[12] and aside from the infrared spectra the rotations are particularly characteristic of this series.

The intermediate eluates represented mixtures of methyl betulinate and methyl oleanolate, but from the last three crystalline eluates there could be isolated after several recrystallizations from methanol 0.45 g. of **methyl oleanolate**, m.p. 199–201°, $[\alpha]^{28}D$ +75°. The separation of methyl betulinate and methyl oleanolate appears to be impossible by crystallization due to mixed crystal formation, and even chromatography on alumina is inefficient.

Isolation of Neutral Triterpenes from *Lemaireocereus hystrix*.—The original alkali-washed ether solution of the triterpene mixture furnished 22.5 g. of brown, semi-solid material which was chromatographed in benzene solution on 300 g. of alumina (AlCOA, grade F-20) deactivated with 12 cc. of a 10% solution of acetic acid in water, 300-cc. fractions being collected. The first six fractions yielded oily material which was discarded. Fractions 7–16 (0.34 g.) after repeated crystallization from chloroform–methanol followed by sublimation at 200° (0.01 mm.) gave colorless crystals with the following constants: m.p. 331–334° (sealed capillary), $[\alpha]^{29}D$ +57.7°, $\lambda_{max}^{CHCl_3}$ 5.66 μ (five-membered lactone), no perceptible color with tetranitromethane.

Anal. Calcd. for $C_{30}H_{46}O_3$: C, 79.24; H, 10.20. Found: C, 79.58; H, 10.50.

The non-identity of this lactone with 18-isoöleanolic acid lactone[13] was demonstrated by the infrared spectrum and optical rotation; furthermore, earlier model experiments[5] had demonstrated that oleanolic acid is not lactonized under the conditions prevailing in the acid hydrolysis of the cactus glycosides. The substance appears to be isomeric with thurberogenin,[3,14] but lack of material precluded any further study.

Fractions 18–37 (benzene–ether, 9:1) were combined and recrystallized six times from acetone and acetone–hexane to furnish 0.9 g. of **erythrodiol** (II), m.p. 229–231.5°, $[\alpha]^{29}D$ +74°, identified with authentic material[4] by means of mixture melting point and infrared comparison.

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.29; H, 11.36.

Erythrodiol diacetate showed m.p. 185–186°, $[\alpha]^{29}D$ +53°; the melting point of a mixture with authentic material was not depressed.

Chromatogram fractions 42–46 (ether) after two crystallizations from acetone yielded a total of 2.33 g. of **longispinogenin (III)**, m.p. 248–250° (Kofler), 253.5–254° (sealed capillary), $[\alpha]^{29}D$ +54°; identity with authentic longispinogenin from *L. longispinus*[4] was established by mixture melting point determination. The analytical sample was sublimed at 200° (0.3 mm.).

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.39; H, 10.94.

Acetylation at room temperature (12 hours) followed by recrystallization from methanol yielded **longispinogenin triacetate**, m.p. 220–222°, $[\alpha]^{29}D$ +71°. The infrared spectrum was identical with that of authentic longispinogenin triacetate.[4]

Anal. Calcd. for $C_{36}H_{56}O_6$: C, 73.93; H, 9.65; CH_3CO, 22.01. Found: C, 73.70; H, 9.59; CH_3CO, 21.99.

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(12) Elsevier's "Encyclopedia of Organic Chemistry," Vol. 14, 1940, p. 571.

(13) D. H. R. Barton and N. J. Holness, *J. Chem. Soc.*, 78 (1952).

(14) The earlier reported (ref. 3) light yellow coloration of thurberogenin with tetranitromethane could not be observed again with perfectly pure thurberogenin and hence must have been due to a small amount of impurity, possibly oleanolic acid.

Improved Synthesis of Amino Acid Benzyl Esters

BY BERNARD F. ERLANGER AND RONALD M. HALL

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Amino acid benzyl esters have been found to be useful intermediates for the synthesis of peptides because of their ability to be converted into acids by hydrogenolysis, enabling one to eliminate saponification procedures. When using the "carbobenzyloxy" method of peptide synthesis,[1] this decreases the number of steps and increases yields. Elimination of the need for saponification could also make possible the synthesis of peptides with alkali labile bonds.

Up to the present, benzyl esters of the following amino acids have been synthesized using various techniques: hydroxyproline,[2] glycine,[3–6] L-cysteine,[3] L- and D-glutamic acid (γ-ester),[7] (α-ester and dibenzyl)[8] L- and D-alanine,[5] L-leucine,[6] DL-phenylalanine,[6] ϵ-carbobenzyloxy-L-and-D-lysine[9] and L-proline.[10] The synthetic methods employed for the preparation of many of the amino acid benzyl esters are indirect and tedious and result in poor yields. It is because of this that the benzyl esters are not more widely used.

We are reporting a procedure which is simple and productive of good yields. It consists of the direct esterification of the amino acid in the presence of polyphosphoric acid[11] as the dehydrating agent. The reaction is carried out at temperatures of 90–105° (depending upon the amino acid) for four hours. We have prepared the benzyl ester hydrochlorides of L-alanine, DL-phenylalanine, L-leucine, L-phenylalanine, L-tyrosine and L-cysteine and L-cystine (by oxidation of L-cysteine benzyl ester). Analytical

(1) For a more complete discussion, see J. S. Fruton, *Advances Protein Chem.*, **5**, 1 (1949).

(2) E. Smith and M. Bergmann, *J. Biol. Chem.*, **153**, 627 (1947).

(3) C. R. Harrington and T. H. Mead, *Biochem. J.*, **30**, 1598 (1936).

(4) R. Ruggli, R. Ratti and E. Henzi, *Helv. Chim. Acta*, **12**, 361 (1928).

(5) B. F. Erlanger and E. Brand, THIS JOURNAL, **73**, 3508 (1951).

(6) H. K. Miller and H. Waelsch, *ibid.*, **74**, 1092 (1952).

(7) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(8) H. Sachs and E. Brand, THIS JOURNAL, **75**, 4610 (1953).

(9) B. F. Erlanger and E. Brand, *ibid.*, **73**, 4025 (1951).

(10) R. E. Neuman and E. Smith, *J. Biol. Chem.*, **193**, 97 (1951).

(11) Kindly supplied as a sample by Victor Chemical Works, Chicago 4, Illinois.

TABLE I

BENZYL ESTER HYDROCHLORIDES

Amino acid	Formula	M.p., °C.	Nitrogen, % Calcd.	Found	$[\alpha]^{25}D$ (0.1 N HCl)[a]	C[b]
L-Alanine	$C_{10}H_{14}O_2NCl$	140[c]	6.5	6.5	−14.3[d]	2.11
L-Leucine	$C_{13}H_{20}O_2NCl$	128[e]	5.4	5.4	− 6.6[f]	2.06
L-Phenylalanine	$C_{16}H_{18}O_2NCl$	203	4.8	4.7	−22.5[g]	1.01
L-Tyrosine	$C_{16}H_{18}O_3NCl$	205	4.5	4.6	−23.3	0.97
L-Cysteine	$C_{10}H_{14}O_2NSCl$	106	5.7	5.5	−26.6	1.01
L-Cystine	$C_{20}H_{26}O_4N_2S_2Cl_2$	166 dec.	5.7	5.5	+32.8	0.68
DL-Phenylalanine	$C_{16}H_{18}O_2NCl$	196	4.8	4.9		

[a] All rotations are calculated as the free ester. [b] Concentration in g./100 ml. for optical rotation measurement. [c] Erlanger and Brand (ref. 5) report 140°. [d] The value reported by Erlanger and Brand (ref. 5) is not calculated as free ester. If calculated as free ester, $[\alpha]^{25}D$ becomes −13.2°. [e] Miller and Waelsch (ref. 6) report 128°. [f] Miller and Waelsch (ref. 6) report $[\alpha]^{30}D$ −8° (2% in 0.1 N HCl). [g] In 0.25 N HCl.

data are presented in Table I. The benzyl ester hydrochlorides of L-phenylalanine, L-tyrosine, L-cysteine and L-cystine have not heretofore been described. The benzyl esters of L-leucine, L-phenylalanine and L-tyrosine were reduced to their respective amino acids which were found to be unracemized. The oxidation of cysteine benzyl ester to cystine benzyl ester was performed with iodine in ethyl alcohol. The yield was not very satisfactory and this cannot be considered the synthetic method of choice. Iodine in acetic acid[12] was just as unsatisfactory.

The presence of a free phenolic group in L-tyrosine benzyl ester hydrochloride was confirmed by a positive Millon test. The presence of a free SH group in the L-cysteine benzyl ester hydrochloride was confirmed by a positive nitroprusside test.

Experimental[13]

Starting Materials.—The specific rotations of the amino acids used are as follows: L-alanine + 14.2 (6 N HCl), L-cysteine +4.3 (N HCl), L-leucine +15.2 (6 N HCl), L-phenylalanine −35.1 (H_2O), L-tyrosine −13.0 (3 N NaOH, T = 18°).

DL-Phenylalanine Benzyl Ester Hydrochloride.—Two grams (0.012 mole) of DL-phenylalanine was added to a large test-tube containing a homogenous solution of 25 ml. of benzyl alcohol and 5 g. of polyphosphoric acid. The mixture was stirred in an oil-bath at 90–95° for four hours. (The DL-phenylalanine dissolves within a few minutes.) The solution was then poured into 200 ml. of water containing about 10 ml. of concentrated HCl. Ether was added and the water layer collected. The ether layer was then washed three times with 2% HCl. All aqueous fractions were collected, brought to a pH of about 10 with solid Na_2CO_3 and shaken with three 100-ml. portions of ether. The ether layer was dried over magnesium sulfate and nearly saturated with HCl gas. The DL-phenylalanine benzyl ester hydrochloride, which precipitated, weighed 2.3 g. (65%). The crude product, m.p. 195–196°, was recrystallized from ethyl acetate–petroleum ether.

All the other benzyl ester hydrochlorides were prepared in the same way and in similar yield as DL-phenylalanine benzyl ester hydrochloride with certain exceptions which will be mentioned below.

L-Alanine benzyl ester hydrochloride: recrystallized from methanol–ether.

L-Leucine Benzyl Ester Hydrochloride.—After the dried ether solution had been saturated with HCl gas, the ether was removed *in vacuo*. The residue was crystallized by dissolving in warm ethyl acetate and carefully adding ligroin. It was recrystallized from chloroform–cyclohexane. Upon hydrogenolysis in the presence of palladium black, the recovered amino acid had $[\alpha]^{25}D$ +14.5° (*c* 2, in 6 N HCl).

L-Phenylalanine Benzyl Ester Hydrochloride.—Hydrogenolysis yielded free amino acid with $[\alpha]^{24}D$ −33.6° (*c* 1.20, H_2O).

L-Tyrosine Benzyl Ester Hydrochloride.—Hydrogenolysis yielded free amino acid with $[\alpha]^{26}D$ −12.6° (*c* 1.35 in 3 N NaOH). The Millon test was positive.

L-Cysteine Benzyl Ester Hydrochloride.—The temperature of the bath during the reaction was 105°. At the end of four hours, about 200 ml. of ether was added to incipient turbidity. The solution was saturated with HCl gas, the walls of the container were scratched and the solution kept in the ice-box for 48 hours. The benzyl ester, which crystallized, weighed 1.35 g. (45% of theory); m.p. 91–97°. It was recrystallized from ethyl acetate. The nitroprusside test for sulfhydryl groups was positive.

L-Cystine Benzyl Ester Hydrochloride.—Two hundred and forty-eight mg. (0.001 mole) of L-cysteine benzyl ester hydrochloride was dissolved in 5 ml. of 60% ethanol. To this solution was added dropwise a N solution of iodine in 95% ethanol until a yellow color persisted (1.95 ml. was necessary). The solvent was removed *in vacuo*, 5 ml. of water was added and the pH brought to about 9.5 with K_2CO_3. It was then shaken twice with 20-ml. portions of ether. The ether solution was dried over magnesium sulfate and then HCl gas passed in. The cystine benzyl ester hydrochloride, which precipitated, weighed 60 mg. (25% yield). It was recrystallized as needles from methanol–water.

NOTE ADDED IN PROOF.—We have succeeded in preparing ε-carbobenzoxy L-lysine benzyl ester hydrochloride.[9] Also, L-phenylalanine benzyl ester hydrochloride may be isolated by adding three volumes of ether to the reaction mixture and saturating with hydrogen chloride gas. (See cysteine benzyl ester hydrochloride.)

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(12) *Cf.* T. W. Rall and A. L. Lehninger, *J. Biol. Chem.*, **194**, 120 (1952); also, R. Kuhn, L. Birkofer and F. W. Quackenbush, *Ber.*, **72**, 407 (1939).

(13) All melting points are corrected.

Nucleophilic Displacement of Groups in Substituted Duryl Phenyl Ketones by the Action of Phenylmagnesium Bromide

BY REYNOLD C. FUSON AND WILLIAM S. FRIEDLANDER[1]

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In previous papers of this series nucleophilic displacement by the action of Grignard reagents on hindered diaryl ketones has been accomplished with halogen, methoxyl, acyloxyl and cyano groups.[2] The present paper reports the result of a study of certain duryl phenyl ketones that have substituents in the *ortho* position of the phenyl radical. Phenylmagnesium bromide has been employed in all the experiments since previous studies had shown that the ease of displacement of groups

(1) Procter and Gamble Company Fellow, 1953–1954.

(2) R. C. Fuson and W. S. Friedlander, THIS JOURNAL, **75**, 5410 (1953).