EXPERIMENTAL

All spectra were determined in pure, distilled methylene chloride using matched 1-cm. glass stoppered absorption cells and a Beckman Model DK-1 recording ultraviolet spectrophotometer.

All 2,4-dinitrophenylhydrazones were prepared from the pure ketones and 2,4-dinitrophenylhydrazine hydrochloride in 50% aqueous ethanol containing hydrochloric acid catalyst and were recrystallized to constant melting point[4] from ethanol.

Dicyclopropyl ketone 2,4-dinitrophenylhydrazone, prepared as described above, was obtained as bright red crystals melting at 193–194°.

Anal. Calc'd for $C_{13}H_{14}N_4O_4$: C, 53.78; H, 4.86. Found: C, 53.78; H, 4.81.

Methyl cyclopropyl ketone 2,4-dinitrophenylhydrazone was obtained as reddish-orange crystals, m.p. 155°.

Anal. Calc'd for $C_{11}H_{12}N_4O_4$: C, 49.99; H, 4.57. Found: C, 50.27; H, 4.84.

Methyl vinyl ketone 2,4-dinitrophenylhydrazone was obtained as orange crystals, m.p. 142°.

Anal. Calc'd for $C_{10}H_{10}N_4O_4$: C, 47.99; H, 4.02. Found: C, 48.13; H, 4.02.

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(4) With the exception of those compounds presented below, all melting points checked those given by R. L. Shriner and R. C. Fuson, *Identification of Organic Compounds*, Second Edition, 1947, John Wiley and Sons, Inc., New York, N. Y.

The Alkylation of Ethyl and *tert*-Butyl Acetates[1]

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The common alkylation of ethyl malonate, accompanied by hydrolysis and decarboxylation, amounts to the indirect alkylation of ethyl acetate in which ethyl malonate would be regarded as α-carbethoxy ethyl acetate. The direct alkylation of ethyl acetate is difficult to achieve because this ester tends to undergo self-condensation when its α-hydrogen is ionized by a base, as would be required for the alkylation. Thus, whereas ethyl malonate shows no tendency to self-condense when its α-hydrogen is ionized by sodium ethoxide in its alkylation, ethyl acetate evidently underwent considerable self-condensation when its α-hydrogen was ionized by sodium triphenylmethide in an attempt to effect its benzylation.[2] The intermediates in these reactions would be I and II respectively.

$NaCHCOOC_2H_5$ (with $COOC_2H_5$) — I

$NaCH_2COOC_2H_5$ — II

(1) Supported by the National Science Foundation.

(2) B. E. Hudson and C. R. Hauser, *J. Am. Chem. Soc.*, 62, 2457 (1940).

In the present investigation ethyl and *tert*-butyl acetates were alkylated in low to fair yields by means of sodium amide in liquid ammonia.

The addition of ethyl acetate to a molecular equivalent of sodium amide in liquid ammonia, followed within a few seconds by an equivalent of benzyl chloride, produced the dibenzylated ester which was isolated in 16% yield as the corresponding acid (IV). No monobenzylated ester, which would have been isolated as acid III, was found. Some acetamide was probably produced although none of it was isolated.

$C_6H_5CH_2CH_2COOH$ (III) $\quad (C_6H_5CH_2)_2CHCOOH$ (IV)

In line with a recent observation[3] that lithium amide when used in excess favors certain condensations involving ethyl acetate, the addition of ethyl acetate to two equivalents of this reagent in liquid ammonia, followed by two equivalents of benzyl chloride, gave a better yield (30%) of alkylation product. Moreover, this product consisted exclusively of the monobenzylated ester which was isolated as the corresponding acid (III).

Since *tert*-butyl acetate not only undergoes relatively more α-hydrogen ionization with an alkali amide than ethyl acetate but also exhibits less tendency to self-condense, the *tert*-butyl ester should be more suitable for alkylation. In agreement with this, the benzylation of *tert*-butyl acetate by means of potassium amide was realized in better yield than that of ethyl acetate by means of sodium amide under similar conditions.[4] Thus, the former alkylation produced a 45% yield of acid IV (after hydrolysis) compared to the 16% yield with ethyl acetate. The intermediate potassio *tert*-butyl acetate (V) was first prepared by means of potassium amide in liquid ammonia.

$KCH_2COOC(CH_3)_3$ (V)

The alkylation of potassio *tert*-butyl acetate (V) with *n*-octyl bromide under similar conditions gave more of the monoalkylated than dialkylated product. The yields of the corresponding acids, which were isolated, were 41% and 12% respectively.

$C_8H_{17}CH_2COOH$ (VI) $\quad (C_8H_{17})_2CHCOOH$ (VII)

EXPERIMENTAL

Benzylation of ethyl acetate. (A) By sodium amide. To a stirred suspension of 0.3 mole of sodium amide in 250 ml. of liquid ammonia[5] was added during one minute 26.4 g. (0.3

(3) C. R. Hauser and J. K. Lindsay, *J. Am. Chem. Soc.*, 77, 1050 (1955).

(4) Both potassium amide and sodium amide gave the same results in the butylation of *tert*-butyl diethylacetate; see C. R. Hauser and W. J. Chambers, *J. Am. Chem. Soc.*, 78, 3837 (1956).

(5) See C. R. Hauser, F. W. Swamer, and J. T. Adams, *Org. Reactions*, 8, 122 (1954).

mole) of ethyl acetate in 50 ml. of anhydrous ether, followed immediately by 38.0 g. (0.3 mole) of benzyl chloride in 25 ml. of anhydrous ether during 1½ minutes. After one hour, the liquid ammonia was evaporated (steam-bath), and ether and water were added. The two layers were separated, and the ethereal layer was dried and the solvent was removed. The residue was distilled *in vacuo* (15 mm.) until the temperature rose to 100°, 50% of the benzyl chloride being recovered. The pot residue then was heated with 10% sodium hydroxide on the steam-bath for 16 hours. The resulting solution was acidified, and the mixture was extracted with ether. The solvent was removed and the residue was crystallized from a mixture of acetone and petroleum ether to give 5.8 g. (16%) of dibenzylacetic acid (IV), m.p. 88–88.5°, reported m.p. 89°.[6]

(*B*) *By lithium amide.* To a stirred suspension of 0.2 mole of lithium amide in 250 ml. of liquid ammonia was added rapidly 8.8 g. (0.1 mole) of ethyl acetate in 20 ml. of anhydrous ether, followed after 2 minutes by 25.3 g. (0.2 mole) of benzyl chloride in 20 ml. of anhydrous ether. After one hour, the liquid ammonia was evaporated (steam-bath) as ether was being added. The resulting ether suspension was refluxed for 15 minutes, and water then was added. The mixture was worked up as described above. The acid, obtained on saponification, was recrystallized from petroleum ether (solution cooled on Dry Ice) to give 4.5 g. (30%) of hydrocinnamic acid (III), m.p. 48.5–49°, reported m.p. 46–47°.[7]

Alkylation of tert-butyl acetate. (*A*) *With benzyl chloride.* To a stirred suspension of 0.1 mole of potassium amide in 250 ml. of liquid ammonia was added 11.6 g. (0.1 mole) of *tert*-butyl acetate in 20 ml. of anhydrous ether to produce, within one-half minute, a white precipitate. After 2 minutes, 13.9 g. (0.11 mole) of benzyl chloride was added during 5 minutes, and the mixture was stirred one hour. The liquid ammonia was evaporated as ether was being added, and the resulting mixture was acidified. The two layers were separated, and the solvent was removed from the ethereal layer. The residue was refluxed with a mixture of dioxane and 37% hydrochloric acid for 3 hours. Ether and water were added, and the ethereal layer was extracted with 10% sodium hydroxide followed by water. The alkaline solution was acidified and extracted with ether. The solvent was removed from the dried ethereal solution, and the residue was recrystallized from acetone and petroleum ether to give 5.6 g. (45%) of dibenzylacetic acid (IV), m.p. 88–88.5°, reported m.p. 89°.[6]

(*B*) *With n-octyl bromide.* This alkylation was effected as described for the benzylation employing 21.1 g. (0.11 mole) of *n*-octyl bromide. The crude acidic product was fractionated *in vacuo* to give 7.1 g. (41%) of decanoic acid (VI), b.p. 149–153° at 10 mm., m.p. 29–30°, reported b.p. 148–150° at 11 mm., m.p. 31.3°,[8] and 1.9 g. (12%) of α-octyldecanoic acid (VII), b.p. 153–157° at 1 mm., m.p. 37–38°, reported b.p. 183–185° at 5 mm., m.p. 35–36°.[9]

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(6) L. W. Jones and A. W. Scott, *J. Am. Chem. Soc.*, **44**, 407 (1922).
(7) E. Schwenk and D. Papa, *J. Org. Chem.*, **11**, 798 (1946).
(8) L. Deffet, *Bull. soc. chim. Belg.*, **40**, 385 (1931).
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Preparation of D and L Forms of Glycyl-β-2-Thienylalanine[1]

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The toxicity of racemic glycyl-β-2-thienylalanine has been reported.[2,3] In order to obtain direct evidence concerning the toxicity of the two optical isomers of this peptide, the D and L forms have been prepared through the action of carboxypeptidase upon carbobenzoxyglycyl-β-2-thienyl-DL-alanine.[4] Only the L form of the peptide was found to be toxic for *Escherichia coli*, strain 9723.

EXPERIMENTAL

Glycyl-β-2-thienyl-D-alanine. Carboxypeptidase was allowed to act upon 9.0 g. of racemic carbobenzoxyglycyl-β-2-thienylalanine[2] in veronal buffer at *p*H 7.5 until all of the L form had been hydrolyzed, as determined by titration.[6] After acidification of the reaction mixture the carbobenzoxyglycine and carbobenzoxyglycyl-β-2-thienyl-D-alanine were extracted into ether; removal of the ether left a solid residue weighing 3.6 g. This mixture was decarbobenzoxylated with phosphonium iodide in glacial acetic acid[6] to yield 1.4 g. of glycyl-β-2-thienyl-D-alanine containing some glycine. The peptide, after recrystallization from hot water, melted[7] at 245–247° and had a specific rotation of $[\alpha]_D^{25}$ −40.1° (20 mg. in 2 ml. of water).

Anal. Calc'd for $C_9H_{12}N_2O_3S$: N, 12.27. Found: N, 12.36.

β-2-Thienyl-L-alanine. The original enzymatic reaction mixture, after acidification and ether extraction, was concentrated to dryness *in vacuo* on a water-bath to leave a residue of β-2-thienyl-L-alanine hydrochloride and other salts. This residue was extracted with warm absolute alcohol, filtered, and the filtrate was neutralized to *p*H 7 with conc'd NH_4OH to precipitate the L amino acid. Yield, 1.6 g.; m.p., 255–256°; specific rotation $[\alpha]_D^{25}$ −31.7° (20 mg. in 2 ml. of water.)[8]

Glycyl-β-2-thienyl-L-alanine. The above β-2-thienyl-L-alanine (1.6 g.) was reacted at 5° with an equivalent amount of chloroacetyl chloride in the presence of two equivalents of *M* NaOH to obtain 1.5 g. of chlorocetyl-β-2-thienyl-L-alanine; m.p., 124°; specific rotation $[\alpha]_D^{25}$ +46.6° (20 mg. in 2 ml. of absolute ethanol).[8] Then 0.7 g. of chloroacetyl-β-2-thienyl-L-alanine was allowed to stand overnight at room temperature in 20 ml. of concentrated ammonium hydroxide. Removal of the solvent and recrystallization of the residue from a mixture of hot ethanol and water yielded 0.6 g. of

(1) This work was supported by research grant RG-2843 from the United States Public Health Service.
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(5) W. Grassman and W. Heyde, *Z. physiol. Chem.*, **183**, 32 (1929).
(6) C. R. Harrington and T. H. Mead, *Biochem. J.*, **29**, 602 (1935).
(7) Melting points were taken on a melting point block and are uncorrected.
(8) B. F. Crowe and F. F. Nord, *J. Org. Chem.*, **15**, 688 (1950).