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]

DEPARTMENT OF CHEMISTRY
DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

(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).
(9) W. M. Stanley, M. S. Jay, and R. Adams, *J. Am. Chem. Soc.*, **51**, 1261 (1929).

Preparation of D and L Forms of Glycyl-β-2-Thienylalanine[1]

FLOYD W. DUNN

Received July 31, 1956

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.
(2) F. W. Dunn and K. Dittmer, *J. Biol. Chem.*, **188**, 263 (1951).
(3) F. W. Dunn, J. M. Ravel, and W. Shive, *J. Biol. Chem.*, **219**, 809 (1956).
(4) F. W. Dunn and E. L. Smith, *J. Biol. Chem.*, **187**, 385 (1950).
(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).

glycyl-β-2-thienyl-L-alanine; m.p. 245–248°; specific rotation $[\alpha]_D^{25}$ +40.0° (20 mg. in 2 ml. of water).

Anal. Calc'd for $C_9H_{12}N_2O_3S$: N, 12.27. Found: N, 12.05.

Microbiological assay. The procedure was identical with that previously reported[3] for *E. coli* 9723. Under these conditions glycyl-β-2-thienyl-L-alanine completely inhibited growth at a level of one microgram in 5 ml. of medium; the D isomer showed no toxicity up to 100 micrograms per 5 ml.

Acknowledgment. The author wishes to thank Dr. Joanne M. Ravel, of the Biochemical Institute of the University of Texas, for the microbiological assay.

DEPARTMENT OF CHEMISTRY
ABILENE CHRISTIAN COLLEGE
ABILENE, TEXAS

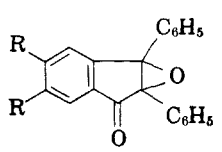
The Rearrangement of 2,3,5,6-Tetraphenylindenone Oxide

HERBERT O. HOUSE, EDWIN A. CHANDROSS, AND BART J. PUMA

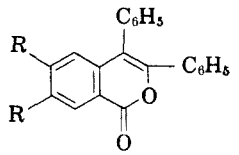
Received July 31, 1956

The acid-catalyzed isomerization of substituted indenone oxide systems (I) has been studied by several groups of workers.[1–4] 2,3-Diphenylindenone oxide (Ia) was reported[1–4] to yield the lactone IIa; however the tetraphenyl compound Ib was reported[3] to yield the red cyclobutanone III, m.p. 219–220°, which could be converted to the colorless lactone IIb, m.p. 230°, by treatment with a variety of reagents. A previous attempt to isolate a cyclobutanone derivative, analogous to III, from the oxide Ia was unsuccessful.[4] As part of a study[5] of isomerizations of α,β-epoxycycloalkanones which result in ring contraction we were led to reinvestigate the behavior of the aforementioned indenone oxides.

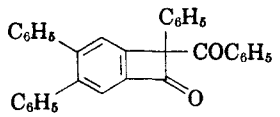
Reaction of the diphenylindenone oxide Ia with a mixture of acetic and sulfuric acids, as previously described,[1–4] produced the lactone IIa. The composition and spectra (Table I) of our product are consistent with the structure previously proposed. Saponification of IIa with ethanolic potassium hydroxide produced the keto acid IVa[1,6] which was reconverted to IIa by treatment with a boiling solution of *p*-toluenesulfonic acid in benzene. The same isomerization product IIa was formed in yields ranging from 60–81% when the oxide Ia was treated with boron trifluoride etherate in either benzene or ether with various reaction times (10–30 minutes). Thus, the isomerization of the oxide Ia in either hydroxylic or non-hydroxylic solvents appears to produce the lactone IIa rather than a cyclobutanone derivative.



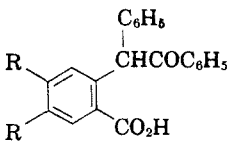
Ia, R = H
b, R = C_6H_5



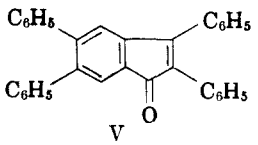
IIa, R = H
b, R = C_6H_5



III



IVa, R = H
b, R = C_6H_5



V

The tetraphenylindenone oxide Ib was prepared by the epoxidation of a solution of the unsaturated ketone V in acetone with alkaline hydrogen peroxide. The unsaturated ketone V was obtained by the thermal rearrangement of 2-bromo-3,3,5,6-tetraphenylindanone as described earlier;[6] in our hands this procedure was definitely superior to an alternate preparative method for V which involved the rearrangement and dehydrogenation of 3,4,5,6-tetraphenylindanone in the presence of sulfur.[7] Recrystallization of either of the products from ethanol afforded two red, crystalline products, m.p. 163–165° and 177–178.5°, rather than the single product, m.p. 166°, previously reported.[7] Both of the products had the composition expected of the ketone V and the infrared and ultraviolet spectra (Table I) of the two samples were identical. Treatment of each of the samples with alkaline hydrogen peroxide yielded the same oxide Ib. We therefore concluded that the two samples represent different crystalline modifications of V.

Samples of the oxide Ib were treated with boron trifluoride in ether, with boron trifluoride etherate in benzene, with a mixture of acetic and sulfuric acids, and with a solution of hydrogen bromide in acetic acid. The last two procedures were reported[3] to convert the oxide Ib to the diketone III. The infrared spectra of the crude, red or orange crystalline products, all of which melted within the range 211–225°, isolated in each case were all essentially identical. Repeated recrystallization of the products from a benzene–hexane or a benzene–ethanol

(1) E. Weitz and A. Scheffer, *Ber.*, **54**, 2327, 2334 (1921).
(2) C. F. Koelsch and C. D. LeClaire, *J. Am. Chem. Soc.*, **65**, 754 (1943).
(3) C. F. H. Allen and J. W. Gates, Jr., *J. Am. Chem. Soc.*, **65**, 1230 (1943).
(4) A. Banchetti, *Gazz. chim. ital.*, **81**, 419 (1951); [*Chem. Abstr.*, **46**, 5022 (1952)].
(5) H. O. House and R. L. Wasson, To be published.
(6) C. F. H. Allen and J. A. Van Allan, *J. Org. Chem.*, **20**, 315 (1955).
(7) C. F. H. Allen and E. W. Spanagel, *J. Am. Chem. Soc.*, **55**, 3773 (1933).