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The Reaction of Hydrazoic Acid with Pinonic Acid and Homoterpenyl Methyl Ketone

BY B. A. PARKIN AND G. W. HEDRICK

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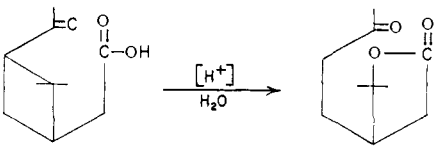
A study of the reactions of *d,l*-pinonic acid, 2,2-dimethyl-3-acetylcyclobutane acetic acid and *d,l*-homoterpenyl methyl ketone, 3-(1-hydroxy-1-methylethyl)-6-ketoheptanoic acid lactone, included an investigation of the behavior of these materials in the Schmidt reaction with hydrazoic acid. Only the reactions involving the methyl ketone group are reported. *d,l*-Homoterpenyl methyl ketone reacted to give a 69.5% yield of a product consisting of a mixture of *d,l*-3-(1-hydroxy-1-methylethyl)-5-acetylaminopentanoic acid lactone (88–90%) and *d,l*-3-(1-hydroxy-1-methylethyl)-5-(N-methylcarboxamide)-valeric acid lactone (10–12%) from which acetic acid, methylamine-*d,l*-homoterpenylic acid and the hydrochloride of the amino acid lactone were isolated and characterized. The free amino acid rearranged readily to the lactam, 4-(1-hydroxy-1-methylethyl)-2,2-piperidone which by reduction yielded a previously reported 4-(1-hydroxy-1-methylethyl)-piperidine. Under the conditions of the Schmidt reaction—concentrated sulfuric acid at low temperature—*d,l*-pinonic acid rearranged readily to give homoterpenyl methyl ketone. Ethyl pinonate behaved similarly. However, with the conditions used *d,l*-pinonic acid and its ethyl ester reacted with hydrazoic acid to give 70 to 75% yield of a mixture of products consisting of *d,l*-2,2-dimethyl-3-acetylaminocyclobutane acetic acid or its ester (85–90%) and N-methylamide of *d,l*-pinic acid or its ester (10–12%) from which acetic acid, methylamine, pinic acid, the hydrochloride of the free amine—2,2-dimethyl-3-aminocyclobutaneacetic acid and the free amine—were isolated and characterized. No rearrangement to give homoterpenyl methyl ketone occurred.

The oxidation of α-pinene by permanganate[1] or ozone[2] results in the formation of pinonic acid (2,2-dimethyl-3-acetylcyclobutaneacetic acid) (I) which, under the influence of mineral acids is converted to homoterpenyl methyl ketone, 3-(1-hydroxy-1-methylethyl)-6-ketoheptanoic acid lactone (II),[3] in nearly quantitative yields. Since these compounds are under investigation in this Laboratory, it was believed desirable to study their behavior to hydrazoic acid in the Schmidt reaction.

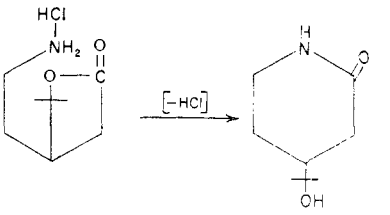


I II

In the present work, the Schmidt reaction with *d,l*-homoterpenyl methyl ketone gave a mixture of products, consisting of about 88–90% of the acetamide of *d,l*-3-(1-hydroxy-1-methylethyl)-5-aminopentanoic acid lactone and 10–12% of N-methylhomoterpenylic amide, *d,l*-3-(1-hydroxy-1-methylethyl)-5-(N-methylcarboxamide)-pentanoic acid lactone.

Hydrolysis of the mixture with hydrochloric acid gave, as the main product, the hydrochloride of *d,l*-3-(1-hydroxy-1-methylethyl)-5-aminopentanoic acid lactone (III). The *p*-nitrobenzamide and α-naphthylurea of the lactone amine were prepared from this hydrochloride. Freeing the lactone amine from its salt resulted in its conversion to *d,l*-4-(1-hydroxy-1-methylethyl)-2-piperidone (IV).

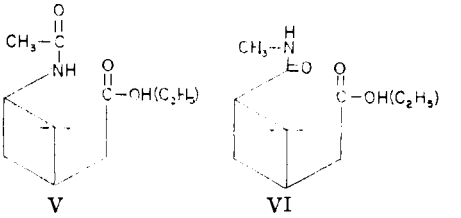
Alkaline hydrolysis of the mixture liberated methylamine which was distilled off, titrated and characterized as the *p*-nitrobenzamide. The methylamine obtained was generally about 10–12 mole per cent. Hydrolysis with acid allowed distillation of the acetic acid which was characterized by the anilide. Although the last traces were difficult to remove, the acetic acid obtained was generally approximately 90 mole per cent. The presence of the N-methylamide of homoterpenylic acid in the product was further shown by the isolation of 8 mole per cent. of homoterpenylic acid from extracts of acid hydrolysates of the original product.



III IV

Conversion of the free lactone amine to the lactam is indicated by the lack of basic character of the material isolated. It was converted back to the hydrochloride by refluxing with hydrochloric acid, but contact with hydrogen chloride in organic solvents did not give the hydrochloride. The material also gave no test for amino nitrogen on treatment with nitrous acid. Reduction of the material with lithium aluminum hydride gave 4-(1-hydroxy-1-methylethyl)-piperidine, a compound previously reported.[4]

The reaction of *d,l*-ethyl pinonate with hydrazoic acid gave a colorless liquid when distilled which consisted of the ethyl esters of *d,l*-2,2-dimethyl-3-acetylaminocyclobutaneacetic acid (V) (88–90%) and 2,2-dimethyl-3-(N-methylcarboxamide)-cyclobutaneacetic acid (VI) (10–12%).



V VI

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(1) M. Delepine, *Bull. inst. pin.*, 174 (1936); *Bull. soc. chim.*, [5] **3**, 1369 (1936).

(2) G. S. Fisher and J. S. Stinson, *Ind. Eng. Chem.*, **47**, 1569 (1955).

(3) C. L. Arcus and G. J. Bennett, *J. Chem. Soc.*, 2627 (1955); J. L. Simonsen and L. N. Owen, "The Terpenes," second edition, Vol. II, Cambridge University Press, 1949, p. 149.

(4) G. R. Clemo and E. Hoggarth, *J. Chem. Soc.*, 41 (1941).

The composition was verified by saponification and isolation of the products. By digesting with 6 *N* sodium hydroxide 10–12 mole per cent. of methylamine (characterized by the *p*-nitrobenzamide) was liberated. Acidification of the saponification mixture with dilute sulfuric acid and distilling resulted in 85–90 mole per cent. of acetic acid. The ether-soluble material from an acid saponification gave a product which proved to be pinic acid, presumably a mixture of *cis*- and *trans*-*d,l*-isomers in an amount equivalent to 9 mole per cent. The dicyclohexylamine salt was prepared and gave no melting point depression with the salt from an authentic sample of a *cis* and *trans* mixture of *d,l*-pinic acid.

The hydrochloride of *d,l*-2,2-dimethyl-3-aminocyclobutaneacetic acid, a hygroscopic solid, was obtained in 87% yield from an ether-extracted aqueous hydrochloric acid hydrolysate by concentration and precipitation with acetone. The free amino acid was prepared in almost quantitative yield from a solution of the hydrochloride with a basic ion exchange resin and by treating a solution of the sulfate with barium hydroxide.

The colorless α-naphthylurea prepared from the free amino acid melted at 188–189°, foamed, solidified and remelted at 226–229°. The amino acid polymerized when heated to or above its melting point. When heated at 250°, a high melting (300°) transparent, amber resin resulted.

The reaction of *cis*-*d,l*-pinonic acid with hydrazoic acid resulted in a viscous, amber colored liquid which when subjected to vacuum distillation polymerized with the elimination of acetic acid. A study of the composition of the liquid by methods used above for the characterization of the ethyl ester showed that the reaction product consisted of the same mixture of acids, 88–90% of the acetylamino and 10–12% of the pinic acid derivatives. The free amino acid, its hydrochloride and α-naphthylureide were identical to that obtained from *d,l*-ethyl pinonate.

Acetylation of the amino acid and esterification with *p*-bromophenacyl bromide gave a product identical to the *p*-bromophenacyl ester from the crude mixture of acids obtained from pinonic acid. The identity of these derivatives proves that acid hydrolysis is not accompanied by molecular rearrangement.

Discussion

The reaction with homoterpenyl methyl ketone proceeded in a normal manner with no accompanying side reactions. On the other hand, with pinonic acid side reactions were expected because of the instability of the cyclobutane ring in acid medium. One of the most obvious reactions of this type is the formation of homoterpenyl methyl ketone, already referred to, by molecular rearrangement of pinonic acid. It was found that under the conditions of the Schmidt reaction with concentrated sulfuric acid rearrangement occurs readily. Ethyl pinonate reacted similarly. Because of this, minimum contact of the reactants *per se* with sulfuric acid was achieved by dissolving in the hydrazoic acid solution prior to addition to the reaction vessel. By this method the extent of formation of homoterpenyl methyl ketone, if any, was slight as evidenced by the character of the products of the reaction.

Other side reactions involving rupture of the pinonic acid cyclobutane ring were excluded because (a) the amine group was a primary amine thus eliminating the possibility of formation of a pyrrolidine derivative through ring enlargement, (b) ring contraction to produce a cyclopropane ring is unlikely since similarly substituted cyclopropane derivatives are unstable in acid medium[5] and (c) there was no unsaturation in the amino acid obtained, thus obviating the possibility of formation of products through simple ring cleavage.

The only literature reference describing the behavior of a cyclobutane ring to the Schmidt reaction is that of Buckman, *et al.*,[6] who have shown that *cis*- and *trans*-1,2-cyclobutanedicarboxylic acids undergo the Schmidt and Curtius reactions to produce *cis*- and *trans*-1,2-diaminocyclobutanes.

It is concluded, since neither a lactone structure nor unsaturation is present in the molecule and that neither ring enlargement nor contraction have occurred, that the reactions with *d,l*-pinonic acid and its ester produced the amino acid described. Furthermore, although the reactions are not identical, the results of Buckman in a way substantiate this conclusion.

The stereochemistry involving an asymmetric carbon atom in the Schmidt reaction has not been as well established as it is in the similar Beckmann, Hoffmann or Curtius reactions. However, there are a few recorded references[6,7] which indicate retention of configuration of the optically active carbon atom during the Schmidt reaction. It is presumed that the amino acid obtained from *cis*-*d,l*-pinonic acid is the *cis* isomer. However, no definite *cis* or *trans* configuration can be assigned to the products since the configurations of the isomers of pinonic acid have not been unequivocally established.[3]

Acknowledgment.—The authors wish to express their appreciation to L. E. Brown, Analytical, Physical Chemical & Physics Section, Southern Utilization Research and Development Division, for the elemental analyses reported in this manuscript.

Experimental

Preparation of Hydrazoic Acid Solutions.—The method of preparation of chloroform solutions of hydrazoic acid used throughout this work is that described by Herbst[8] using chloroform instead of benzene as a solvent. The reactions of the ketones with hydrazoic acid and sulfuric acid were carried out by method three of Wolff.[9]

Isolation of Products.—After completion of the Schmidt reaction the mixture was neutralized while maintaining the temperature below 50°, either by external cooling or by first diluting with ice and water as needed. When pinonic acid was the reacting substance, the final *p*H was adjusted to 3 to allow the extraction of the free carboxylic acid. This resulted in a clear chloroform layer containing only a small part of the desired products and a third layer which was

(5) John Simonsen, *J. Chem. Soc.*, **115**, 306, 909 (1929); **119**, 1223 (1933); **119**, 1225 (1935).

(6) E. R. Buckman, A. O. Reims, T. Skei and M. J. Schlatter, THIS JOURNAL, **64**, 2696 (1942).

(7) J. V. Braun *Ber.*, **66***B*, 684 (1933); D. W. Adamson, *J. Chem. Soc.*, 1564 (1939).

(8) W. L. Garbrecht and R. M. Herbst, *J. Org. Chem.*, **18**, 1003 (1953).

(9) Hans Wolff, *Org. Reactions*, **3**, 328 (1946).

TABLE I

MIXED AMIDES[a] FROM SCHMIDT REACTION ON *d,l*-HOMOTERPENYL METHYL KETONE, *d,l*-PINONIC ACID AND *d,l*-ETHYL PINONATE

Reacting substance	Yield, %	B.p. °C.	B.p. Mm.	Carbon, % Found	Carbon, % Calcd.	Hydrogen, % Found	Hydrogen, % Calcd.	Nitrogen, % Found	Nitrogen, % Calcd.
d,l-Homoterpenyl methyl ketone[b]	69.5	207	1	60.55, 60.62	60.28	8.56, 8.55	8.60	7.04, 7.06	7.03
dl-Ethyl pinonate	92	153–156	0.2	64.00, 63.74	63.40	9.24, 9.14	9.30	5.57, 5.53	6.16
d,l-Pinonic acid[c]	89								

[a] Values in this table are for products containing both isomeric amides normally formed in the reaction. [b] M.p. 64–67°. [c] M.p. 105–106°.

TABLE II

ANALYSES OF PRODUCTS AND DERIVATIVES FROM THE SCHMIDT REACTION OF *d,l*-PINONIC ACID, *d,l*-ETHYL PINONATE AND *d,l*-HOMOTERPENYL METHYL KETONE

No.		M.p., °C.[b]	Carbon, % Found	Carbon, % Calcd.	Hydrogen, % Found	Hydrogen, % Calcd.	Nitrogen, % Found	Nitrogen, % Calcd.
1	*d,l*-3-(1-Hydroxyl-1-methylethyl)-5-aminopentanoic acid lactone hydrochloride[a]	180.5–181.5						
2	*p*-Nitrobenzamide of lactone amine from 1	77.1–78.0	57.87, 58.03	58.80	5.70, 5.80	5.92	8.92, 8.95	9.15
3	α-Naphthylurea of lactone amine from 1	153.0–154.5	70.20, 70.18	69.98	6.76, 6.90	6.79	8.58, 8.49	8.58
4	*d,l*-4-(1-Hydroxy-1-methylethyl)-2-piperidone	130.6–131.2	61.37, 61.30	61.13	9.54, 9.44	9.62	8.93	8.91
5	4-(1-Hydroxy-1-methylethyl)-2-piperidine (lit. 136°)	135.0–136.6	67.41, 67.32	67.08	12.15, 12.04	11.96	9.85, 9.79	9.78
6	*d,l*-2,2-Dimethyl-3-aminocyclobutaneacetic acid hydrochloride	167.4–168.2	49.79, 49.85	49.60	8.21, 8.33	8.33	7.22, 7.23	7.24
7	*d,l*-2,2-Dimethyl-3-aminocyclobutaneacetic acid	240–242[c]	61.29, 61.25	61.50	9.64, 9.64	9.56	8.88, 8.88	8.92
8	*p*-Bromophenacyl ester, acetamide of 7	166.0–166.8	54.61, 54.63	54.60	5.65, 5.55	5.60	3.52, 3.52	3.54
9	α-Naphthylurea of 7	188–189 and 226–229	70.5	69.74	7.03	6.076	8.57, 8.58	8.59

[a] Found: Cl, 18.20, 18.27; neut. equiv. in presence of formaldehyde, 194.6. Calcd.: Cl, 18.37; neut. equiv., 193.7. [b] Uncorrected. [c] Melts and sublimes; sublimed material.

combined with the chloroform layer and extracts. The solvent was removed by distillation leaving an amber colored viscous liquid. Attempts to isolate the material by distillation resulted in polymerization with the elimination of acetic acid.

The amount of material in the liquid was calculated from titration values using the theoretical molecular weight of 199. Only organic acidity was used in the calculations which was obtained by using the equivalents of sodium hydroxide required for titration of an aliquot between methyl orange and phenolphthalein end-points. The concentration of organic acid in the residue found by this method was in close agreement with that obtained by precipitation of the acid from acetone as dicyclohexylamine salts. For this a few drops of dicyclohexylamine was added to a small aliquot (1 g.) of residue dissolved in acetone. A colorless crystalline salt precipitated, which was isolated, dried and weighed. Attempts to recrystallize the salt to obtain pure products were not successful.

When homoterpenyl methyl ketone or ethyl pinonate was the reacting substance the final *p*H was not critical and was usually taken to about 8. With these, neutralization is not necessary; however, some improvement in yield results by so doing. The materials were isolated by chloroform extraction and evaporation of the combined extracts and original chloroform layer giving the mixture of products listed in Table I.

Hydrolysis of Products from *d,l*-Homoterpenyl Methyl Ketone.—A sample (26 g.) of distilled product from the Schmidt reaction on homoterpenyl methyl ketone was heated at reflux with 40 ml. of 6 *N* hydrochloric acid for approximately 24 hr. The mixture was cooled and thoroughly extracted with ether. The extracted aqueous solution was then evaporated under aspirator vacuum. The residue weighed 23.32 g., a yield of slightly under 100% if the 10–12% of N-methylamide is taken into account. Recrystallization from acetone was carried out with little loss by reworking the liquors. The recrystallized amine hydrochloride, by Sørensen's formal titration, had a neutral equivalent of 194.6 (calcd. 193.7). The *p*-nitrobenzamide and α-naphthylurea of the lactone amine were prepared from the hydrochloride by treatment of the salt with 25% sodium hydroxide and the proper reagent. Physical constants and analyses of these derivatives and the hydrochloride are given in Table II, items 1, 2 and 3.

The ether extract of the hydrolysis mixture of 10 g. of product from homoterpenyl methyl ketone was mixed with 20 ml. of *p*-cymene and set up for distillation. All material distilling below *p*-cymene and about half the *p*-cymene were removed to eliminate acetic acid. The pot residue was treated with an excess of sodium carbonate and thoroughly extracted with ether to remove all non-acidic materials. The aqueous solution was then acidified and again thoroughly extracted with ether. Evaporation of the ether extract gave white crystalline homoterpenylic acid reported by Simonsen[10] weighing 1.5 g., a yield of about 8 mole per cent., m.p. 99.6–101.6°, (lit. 100–102.5), neut. equiv. 184.9–185.0 (calcd. 186), sapn. equiv. 92.8 (calcd. 93).

***d,l*-4-(1-Hydroxy-1-methylethyl)-2-piperidone.**—A sample of recrystallized hydrochloride of *d,l*-2-(1-hydroxy-1-methylethyl)-5-aminopentanoic acid lactone was dissolved in 25–30 ml. of alcohol, treated with the calculated amount of 50% sodium hydroxide, poured into a Büchner filter, and washed with several 10-ml. portions of alcohol. Evaporation of the alcohol and recrystallization from acetone gave 10.38 g. (79% yield) of purified lactam. The filtrate was evaporated and the residue recrystallized from a small quantity of acetone yielding another 1.17 g. of lactam, bringing the yield to 82.5%. Physical constants and analyses are listed in Table II, item 4.

4-(1-Hydroxy-1-methylethyl)-piperidine.—Eleven grams of *d,l*-4-(1-hydroxy-1-methylethyl)-2-piperidone was placed in a 300-ml. flask with an ether solution containing lithium aluminum hydride in excess of one mole per mole of lactam. The lactam is sparingly soluble in the ether solution. The mixture was stirred and heated at reflux for about 36 hr.

(10) John Simonsen, *J. Chem. Soc.*, **91**, 184 (1904).

Ethyl acetate was then added dropwise until the unreacted lithium aluminum hydride was decomposed. Several milliliters of 50% NaOH was added, and then water was added dropwise until the solid hydroxides became granular in appearance. The ether layer was poured off and the solid residue was washed with ether. The combined ethereal solution was evaporated and the residue was sublimed on the steam-bath. White needles of 4-(1-hydroxy-1-methylethyl)-piperidine were obtained in about 32% yield. Analyses and physical constants are given in Table II, item 5.

Products from *d,l*-Pinonic Acid and *d,l*-Ethyl Pinonate.—The *d,l*-pinonic acid, m.p. 105–106°, used in the reaction is reported to be the *cis* isomer.[1] This material was used to prepare *d,l*-ethyl pinonate, b.p. 129° (5 mm.), $n^{20}D$ 1.4532, by direct esterification in carbon tetrachloride using *p*-toluenesulfonic acid as a catalyst. The ester is believed to be a mixture of *cis* and *trans* isomers, although this was not established (see Argus[3]).

The material from the reaction of ethyl pinonate was an amber colored liquid after removal of the solvent in vacuum. Distillation usually gave a small forecut of unreacted ester with little still residue. The main fraction was a colorless liquid, d^{20} 1.0420, $n^{20}D$ 1.4704; MD found 60.9, calcd. 61.11.

Hydrolysis of Materials from *d,l*-Pinonic Acid or its Ester.—Fifty grams of distilled material from the ester (a) or an equivalent amount of material, based on organic acidity, from pinonic acid (b) were placed in a flask with 45 ml. of concentrated hydrochloric acid and 150 ml. of water. The mixture was refluxed 16 hr. and extracted with ether to remove any non-basic material. The aqueous phase was then evaporated to near dryness under aspirator vacuum. Dilution of the residue with acetone resulted in the precipitation of a slightly less than quantitative yield, taking into account the N-methylamide originally present, of the hydrochloride of 2,2-dimethyl-3-aminocyclobutaneacetic acid and methylamine hydrochloride which can be purified by dissolving in a small amount of water or aqueous 50% alcohol and reprecipitating with acetone. Physical constants and analyses are listed in Table II, item 6.

Evaporation of the ether extracts from three hydrolyses of (a) and distillation of the residue gave three fractions. The first, b.p. 77°, was characterized by odor as ethyl acetate, the second was principally acetic acid; and a third, 11 g., b.p. 185–198° (1 mm.), neut. equiv. 97. The viscous distillate was slow to crystallize and was believed to be a mixture of *cis*- and *trans-d,l*-pinic acids. A few drops dissolved in acetone gave a colorless precipitate, m.p. 151–153.5°, by the addition of a few drops of dicyclohexylamine. A salt, m.p. 152.6–153.8°, was prepared similarly from an authentic sample of a mixture of *cis*- and *trans-d,l*-pinic acid, neut. equiv. 97, obtained by the hypohalite oxidation of a pure *cis-d,l*-pinonic acid. A mixture of the salts melted at 152–153.8°. From this evidence the third fraction must be principally pinic acid and the yield is approximately 9 mole per cent. The ether extracts from hydrolyses of (b) gave the same results except no ethyl acetate was obtained.

Saponification of either (a) or (b) with an excess of 6 *N* sodium hydroxide liberated 10 to 12 mole per cent. of methylamine which was recovered by steam distillation and characterized by its *p*-nitrobenzamide. Acidification of the hydrolysate with sulfuric acid and distilling liberated 85 to 90 mole per cent. of acetic acid which was converted to acetanilide for identification. With both the amine and acetic acid dry salts were prepared in order to concentrate the materials for use in making the derivatives. The yields, however, are based upon titration of aliquots of each of the distillates.

Hydrolyses of both (a) and (b) also were carried out using an equivalent amount of 3 *N* sulfuric acid in place of the hydrochloric acid. The resulting hydrolysate, after ether extraction, contained the sulfates of the amino acid and methylamine. However, these could not be isolated in the solid form.

***d,l*-2,2-Dimethyl-3-aminocyclobutaneacetic Acid.**—The amino acid *d,l*-2,2-dimethyl-3-aminocyclobutaneacetic acid was prepared from the crude hydrochloride by use of an ion exchange resin.[11] Evaporation of the eluent resulted in a thick paste having a slight odor of methylamine. Dilution with acetone brought about precipitation of crystalline *d,l*-2,2-dimethyl-3-aminocyclobutaneacetic acid in 90% yield. This is presumably the *cis* isomer. No evidence of a second isomer has been observed.

When sulfuric acid was used for hydrolysis the aqueous solution was treated with sufficient barium hydroxide to precipitate the sulfate ion present. After removal of the barium sulfate by filtration, the amino acid was isolated as above.

Purification of the acid can be achieved by dissolving in a small amount of water, treating with activated charcoal and reprecipitating with acetone or by sublimation at the melting point. At this temperature, however, polymerization also occurs.

The amino acid reacts with nitrous acid at room temperature, liberating a gas which is undoubtedly nitrogen. No attempt was made to characterize the products from this reaction.

The α-naphthylurea and acetamide of the amino acid were prepared by treating with the appropriate reagent. The urea was filtered to remove insolubles and was isolated from the filtrate by acidification. Recrystallization from 50% aqueous ethanol gave a neutral equivalent of 326 which is the theoretical value. The *p*-bromophenacyl ester was prepared from the acetamide by treating an alkaline solution with *p*-bromophenacyl bromide. This product separated out as formed and proved to be identical to the *p*-bromophenacyl ester formed directly from the crude liquid residue isolated from the reaction of *d,l*-pinonic acid and hydrazoic acid. The physical constants and analyses of the amino acid and its derivatives are given in Table II, items 7, 8 and 9.

(11) C. Y. Meyers and L. E. Miller, *Org. Syntheses*, **32**, 13 (1952).

OLUSTEE, FLA.

COMMUNICATIONS TO THE EDITOR

RACEMIZATION BY THE DICYCLOHEXYLCARBODIIMIDE METHOD OF PEPTIDE SYNTHESIS

Sir:

N,N'-Dicyclohexylcarbodiimide was introduced by Sheehan and Hess[1] in 1955 as a peptide-forming reagent. Its ready availability, simplicity of use under mild conditions with frequent good yields of peptides, and the apparent lack of racemization in the formation of optically active peptide derivatives has resulted in widespread use. We have found that racemization can occur, and recommend caution in the use of this reagent. In harmony with our results, which are based on actual separation of isomers, enzymatic evidence has recently been given for racemization in the synthesis of another peptide.[2]

In 1952, a sensitive test for racemization was devised in our laboratories.[3] This involved the reaction of carbobenzoxyglycyl-L-phenylalanine with ethyl glycinate, and subsequent fractional crystallization of the tripeptide product from a 2% solution in ethanol. The racemic form crystallizes first, and careful removal of fractions from time to

(1) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

(2) K. Hofmann, M. E. Woolner, G. Spühler and E. T. Schwartz, *ibid.*, **80**, 1486 (1958).

(3) G. W. Anderson and R. W. Young, *ibid.*, **74**, 5307 (1952); G. W. Anderson, J. Blodinger and A. D. Welcher, *ibid.*, **74**, 5309 1952); J. R. Vaughan, Jr., *ibid.*, **74**, 6137 (1952).