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).

time can detect the presence of less than 1%. Some of the L-form will then subsequently crystallize, the remainder on concentration. The melting point and optical rotation of this material are not absolute criteria of optical purity, since other impurities may be present. This may account for the lack of detection of racemization in the synthesis of the same peptide by Sheehan and Hess.

Following the conditions of Sheehan and Hess[1] as closely as possible, we treated 0.010 molar quantities of carbobenzoxyglycyl-L-phenylalanine (carefully recrystallized from water; m.p. 127.5° (sharp), $[\alpha]^{24}D$ +38.8° ± 0.5° (*c*, 5; ethanol))[4] and ethyl glycinate (freshly distilled) in the presence of 0.011 mole of N,N'-dicyclohexylcarbodiimide[5] in 50 ml. of dry tetrahydrofuran at room temperature. The temperature of the mixture spontaneously rose to 37°, then fell. After four hours, acetic acid was added to decompose excess reagent. Dicyclohexylurea was removed here and after concentrating the filtrate *in vacuo* to about 10 ml. (89.4% yield). Addition of 50 ml. of water and chilling precipitated the tripeptide; it was washed with 2 × 10 ml. of water, 10 ml. of 5% potassium bicarbonate solution, then 2 × 10 ml. of water. The crude dry yield was quantitative, and the m.p. 106–109°. A 2% solution of the product in absolute alcohol gave crystallization on refrigeration (0°). During several hours, fractions of the DL-tripeptide, m.p. in the 129–133° range (the pure compound melts at 132–133°) amounting to 6.6% were collected. After a fraction of a few mg. in the 120–130° range, the L-form began to appear; concentration of the filtrates gave a total of 76% yield, m.p. 116.5–119.5°, $[\alpha]^{25}D$ −11.5°, (*c*, 2, ethanol).[6,7] Two repetitions of the synthesis gave yields of 7.6% DL, 74% L and 8.2% DL, 69% L. For comparison, the reaction was performed in 7 ml. of tetrahydrofuran with tetraethyl pyrophosphite as the reagent, and at reflux temperature for 30 minutes, giving 4.39 g. of crude product, m.p. 118.5–119.5°. Crystallization from 2% solution in ethanol gave no DL form, and 4.24 g. (96% yield) of L-, m.p. 120–120.5°, $[\alpha]^{25}D$ −13.2° (*c*, 2, ethanol).

An experiment with dicyclohexylcarbodiimide at −5° for 48 hours gave 0.5% DL and 75% L of the tripeptide, and another in methylene chloride at room temperature gave 12% DL and 75% L. Thus, temperature and solvent are factors in racemization.

(4) K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **134**, 225 (1940), give m.p. 125–126°, $[\alpha]^{24}D$ +38.5° (*c*, 5, EtOH).

(5) Purchased from Aldrich Chemical Co.

(6) Rotations reported here were obtained by W. Fulmor and staff. The statistical deviation for the tripeptide is ±1°.

(7) Ref. (1) gives m.p. 118–119°, $[\alpha]^{27}D$ −13.5° [ethanol].

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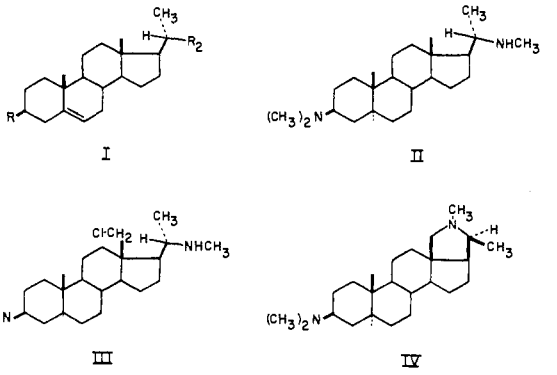
THE SYNTHESIS OF DIHYDROCONESSINE. A METHOD FOR FUNCTIONALIZING STEROIDS AT C_{18}

Sir:

The selective introduction of a functional group in place of hydrogen at a carbon atom not directly joined to labilizing centers such as C=O, C=C, *etc.*, poses an interesting challenge in synthetic chemistry, which is magnified by the rife occurrence of such transformations in Nature under the influence of enzymes. In the field of steroids the problem assumess pecial trenchency with regard to the C_{18} (C/D fusion) angular methyl group because functionality at this position is a special feature of the important hormone, aldosterone, and of the *Holarrhena* alkaloids.[1] This communication describes an efficient and selective method for functionalizing C_{18}, the free radical chain decomposition of an N-chloro-20-aminosteroid in acid solution, and the illustrative synthesis of dihydroconessine (IV).

3β-Acetoxy-20α-aminopregnene-5 acetate[2] (I, R = AcO, R_2 = NH_2·HOAc) was formylated to give 3β-acetoxy-20α-formamidopregnene-5 (I, R = AcO, R_2 = NHCHO), m.p. 191–193°, $[\alpha]^{16}D$ −66°, found: C, 74.32; H, 9.87, which was hydrolyzed to 3β-hydroxy-20α-formamidopregnene-5, m.p. 229–230° (dec.), $[\alpha]^{24}D$ −70.4°, found: C, 76.31; H, 10.08; N, 4.32. Treatment of the hydroxyformamide with *p*-toluenesulfonyl chloride–pyridine yielded crude 3β-tosyloxy-20α-isocyanopregnene-5,[3] which readily was hydrated to 3β-tosyloxy-20α-formamidopregnene-5 (I, R = OTs, R_2 = NHCHO), m.p. 132–133°, $[\alpha]^{27}D$ −47.4°, found: C, 69.92; H, 8.32. 3β-Dimethylamino-20α-formamidopregnene-5, m.p. 226–230° (dec.), $[\alpha]^{27}D$ −52°, found: C, 77.31; H, 10.90; N, 7.23, obtained from the tosylate and dimethylamine, was reduced ($LiAlH_4$) to 3β-dimethylamino-20α-methylaminopregnene-5, m.p. 123–124.5°, $[\alpha]^{27}D$ −37°, found: C, 80.23; H, 11.84; N, 7.78, which on hydrogenation gave 3β-dimethylamino-20α-methylaminoallopregnane (II), m.p. 103.5–104.5°, $[\alpha]^{27}D$ +27.5°, found: C, 79.23; H, 12.06; N, 7.80. 20-N-Chloro-II, prepared using N-chlorosuccinimide in ether,[4] upon irradiation (in 90% H_2SO_4) with ultraviolet light was converted to 3β-dimethylamino-18-chloro-20α-methylaminoallo-pregnane[5]



I II III IV

(1) See R. Tschesche and A. C. Roy, *Ber.*, **89**, 1288 (1956).

(2) P. L. Julian, E. W. Meyer and H. C. Printy, THIS JOURNAL, **70**, 887 (1948).

(3) See W. R. Hertler and E. J. Corey, *J. Org. Chem.*, in press, for other formamide → isocyanide conversions.

(4) H. Ruschig and J. Schmidt-Thomé, U. S. Patent 2,697,107 (1954).

(5) The general intermediacy of δ-chloro-secondary amines in the N-chloroamine → pyrrolidine process has been demonstrated by experiments which will be published separately. *Cf.* W. R. Hertler, Ph.D. Thesis, University of Illinois, 1958, and S. Wawzonek, M. F. Nelson, Jr., and P. J. Thelen, THIS JOURNAL, **73**, 2806 (1951).