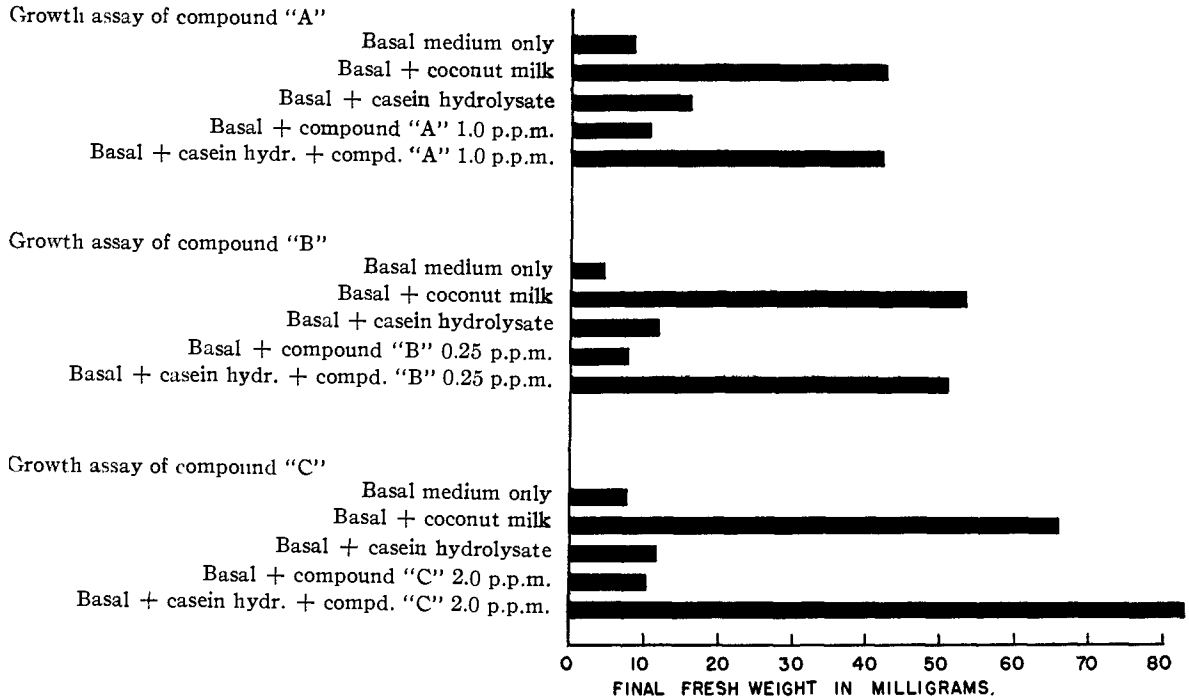

Fig. 6.—Tissue culture growth assays of compounds A, B and C. Each bar represents the average weight of 4 carrot phloem explants after a 14 day test period. Original weight of each explant = 2.6 mg. These tests were not done concurrently and therefore each of the three groups depicts the growth of explants from a different carrot root.

lose column partition chromatography procedure. These fractions (210 mg.) were dissolved in 50 ml. of hot acetone, the volume reduced to 10 ml. and the solution stored for 2 days at −20°. Clusters of prismatic white crystals were obtained which were filtered, washed with cold acetone, recrystallized twice at room temperature from 5 ml. of hot acetone, washed, and dried under vacuum giving a final yield of 19.9 mg.

When determining the melting point it was observed that the material sublimed at 200–210° and condensed in a crystalline state on the cool portions of the capillary. By ultraviolet and infrared absorption, the sublimed material was found to be apparently unchanged and its biological activity in the growth test also withstood this procedure.

The ultraviolet absorption curve of compound C in acid and alkaline solution is shown in Fig. 4. In alkaline solution the absorption maximum at 263 mμ is markedly depressed but unchanged in position. The infrared absorption curve is shown in Fig. 5.

An elementary analysis (single determinations only) showed C, 56.42; H, 8.11; N, 7.67. The growth promoting power of this substance in the carrot phloem explant tissue culture test is shown in Fig. 6.

In summary, the growth-promoting qualities of coconut milk are due in part to a substance or group of substances replaceable by casein hydrolysate. Over and above this, however, there are distinct substances, not contained in casein hydrolysate, which do not appear to be identical with other known vitamin-like compounds. Three such substances have been isolated in crystalline form and the almost certain occurrence of several others has been detected through the use of a carrot tissue culture bioassay procedure. The coconut milk growth factor (C.M.F.) is, therefore, not a single substance but a number of substances, possibly closely related, the identity of which still remains unknown. In view of their dramatic ability to incite random cell division in plant tissues, the isolation of these substances in greater quantity is now being undertaken and their nature and interactions with casein hydrolysate investigated further.

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The Composition of "Cycloheptanol" Produced by the Demianov Rearrangement

BY PETER A. S. SMITH AND DONALD R. BAER

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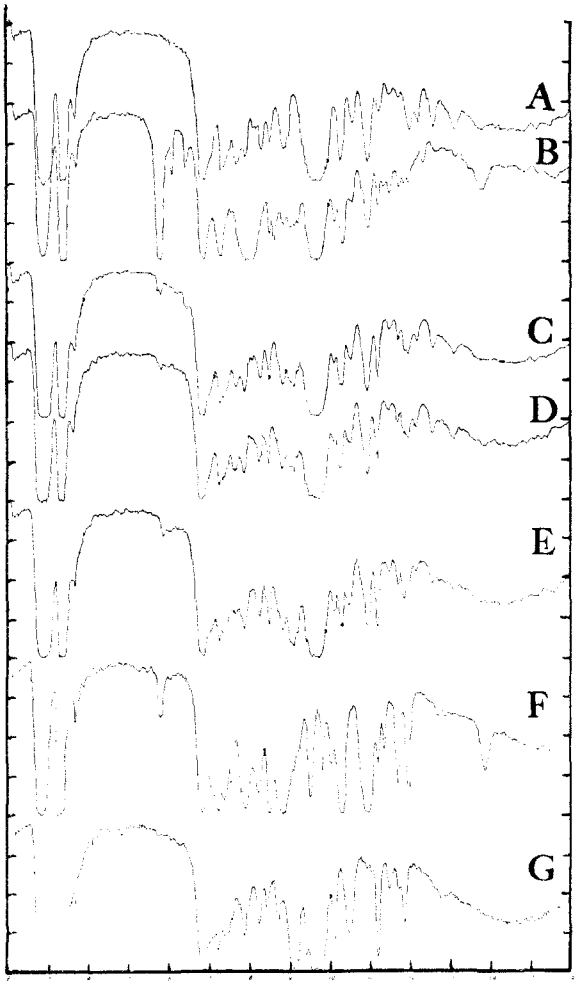
The Demianov rearrangement of hexahydrobenzylamine with nitrous acid has been used as an example[1] of facile ring-expansion, and the product of the reaction has been and is being used as an intermediate in synthesis with the explicit assumption that it is pure cycloheptanol, free from other substances of similar boiling point.[2] We have found that the product consists surely of four and perhaps six, components, of which cycloheptanol constitutes not more than 65%. The other components are cyclohexylcarbinol, 1-methylcyclohexanol and the acetates of one or all of these alcohols. The sequestering of much of the product as acetate is presumably responsible for the failure of Ruzicka and Brugger to detect the cyclohexylcarbinol by reaction with phthalic anhydride.[2]

We have prepared ester-free "cycloheptanol" using sodium dihydrogen phosphate instead of acetic acid; its infrared absorption spectrum (Fig. 1, C) differs from that (Fig. 1, B) of the product ob-

(1) For example, see R. C. Fuson in H. Gilman's "Treatise on Organic Chemistry," Vol. I, John Wiley and Sons, New York, N. Y., 1943, p. 97.

(2) L. Ruzicka and W. Brugger, *Helv. Chim. Acta*, **9**, 399 (1926).

tained by Ruzicka and Brugger's method by the absence of the strong band at 5.8 μ due to carbonyl absorption from the presence of about 30% of ester. A presumably pure reference sample of cycloheptanol was prepared by the lithium aluminum hydride reduction of the heart-cut of a 400-ml. sample of suberone.[3] In the spectra of both samples produced by the Demianov reaction there are bands, notably at 8.5, 9.1 and 11.2 μ, not present in the spectrum of pure cycloheptanol (Fig. 1, A). These bands appear in the spectra of cyclohexylcarbinol (Fig. 1, G) and 1-methylcyclohexanol (Fig. 1, F), and from a synthetic mixture of 62% cycloheptanol, 33% cyclohexylcarbinol and 5% 1-methylcyclohexanol a nearly exact match (Fig. 1, D) is obtained for the spectrum of "cycloheptanol" produced in phosphate solution. The "cycloheptanol" produced in acetate solution after being freed of ester by saponification[4] gives a spectrum (Fig. 1, E) showing only small, quantitative differences (estimated proportions 50:40:10). In confirmation of these results, the permanganate oxidation of the Demianov "cycloheptanol" yielded cyclohexanecarboxylic acid, isolated as its anilide.



Wave length in microns.

Fig. 1.—Infrared absorption spectra, undiluted liquids, Baird spectrophotometer: A, cycloheptanol; B, Demianov "Cycloheptanol" prepared with acetic acid; C, as B, but prepared with sodium dihydrogen phosphate; D, 63% cycloheptanol, 32% cyclohexylcarbinol, 5% 1-methylcyclohexanol; E, as B, after saponification; F, 1-methylcyclohexanol; G, cyclohexylcarbinol.

(3) Kindly supplied by Dr. F. F. Blicke and Mr. Juan Azuara.

Inasmuch as the alcohols and their acetates all boil close to each other, the Demianov rearrangement should no longer be taken as a reliable route to cycloheptanol and the purity of substances already prepared from such "cycloheptanol" should be held in doubt.

Experimental

Hexahydrobenzylamine.—To a solution of 142 g. (1 mole) of cyclohexylacetic acid in 320 ml. of concd. sulfuric acid, heated to 50° and covered by about a half-inch layer of chloroform, was added 80 g. (1.25 moles) of sodium azide in portions with stirring, at such a rate as to keep the temperature between 50 and 60°. The mixture was then heated on a steam-bath for 30 minutes and poured onto 1200 g. of ice. The resulting aqueous solution was cautiously alkalized with concd. sodium hydroxide solution and the amine layer was separated and dried over potassium hydroxide. The distilled product, b.p. 159–161° (740 mm.), weighed 99.4 g. (88%).[5]

Cycloheptanol.—A 7.7-g. sample of suberone, b.p. 68–70° (18 mm.), was reduced with 0.92 g. of lithium aluminum hydride according to the standard procedure of Nystrom and Brown.[6] The product distilling at 87–88.5° (18 mm.) was collected as cycloheptanol; wt. 3.9 g. (50%), $n^{20}D$ 1.4757.[7]

Hexahydrobenzyl Alcohol.—The reduction of 15 g. of cyclohexanecarboxylic acid with lithium aluminum hydride gave 6.8 g. (56%) of alcohol, b.p. 181–182° (740 mm.).[8]

1-Methylcyclohexanol was prepared by the action of methylmagnesium iodide on cyclohexanone.[9] The weak absorption band at 5.8μ in its infrared spectrum (Fig. 1, F) indicates the presence of traces of ketone.

"Cycloheptanol" from Hexahydrobenzylamine. A. In Acetic Acid Solution.—The procedure of Ruzicka and Brugger[2] was applied to 10.0 g. (0.088 mole) of amine, and yielded 2.5 g. of olefins, b.p. 85–120° (740 mm.) and 6.0 g. of alcohols, b.p. 145–180° (740 mm.); 0.3 g. of unreacted amine was recovered. This material gave infrared spectrum B (Fig. 1); repeated fractional distillation reduced the intensity of the carbonyl absorption band (5.8 μ), but a carbonyl-free material could not be thus obtained.

B. **In Phosphate Solution.**—A saturated aqueous solution of 7.1 g. of sodium nitrite (0.11 mole) was added to a solution of 11.3 g. (0.10 mole) of amine and 55 g. (0.4 mole) of sodium dihydrogen phosphate in 150 ml. of water, and heated on a steam-bath for four hours; a brown oil separated. The mixture was then steam distilled and the distillate was extracted with three portions of ether. The combined extracts were fractionally distilled after drying over magnesium sulfate and yielded 2.6 g. (27%) of olefins, b.p. 95–104° (740 mm.), and 5.2 g. (46%) of alcohols, b.p. 158–184° (740 mm.), 2.2 g. (15%) of amine was recovered. The alcohol fraction gave infrared spectrum C (Fig. 1).

Oxidation of Saponified "Cycloheptanol."—Ten grams of the alcohol mixture (footnote 5) was stirred overnight with a solution of 11.4 g. of potassium permanganate in 225 ml. of

(4) This was done by Mr. Juan Azuara by refluxing 57 g. of "cycloheptanol" overnight with alcoholic potassium hydroxide, which yielded 28.8 g. of distilled alcohols, b.p. 178–184° (740 mm). His "cycloheptanol" was an aliquot of the distilled product obtained from 5 moles of amine, 5 moles of acetic acid and 6 moles of sodium nitrite.

(5) O. Wallach, *Ann.*, **353**, 299 (1907), reports b.p. 162–164°.

(6) R. F. Nystrom and W. G. Brown, THIS JOURNAL, **69**, 1197 (1947).

(7) I. Vogel, *J. Chem. Soc.*, 1336 (1938), reports $n^{20}D$ 1.4747; J. Böeseken and C. J. A. Hanegraaff, *Rec. trav. chim.*, **61**, 69 (1942), report $n^{20}D$ 1.4753; L. Ruzicka, P. A. Plattner and H. Wild, *Helv. Chim. Acta*, **28**, 395 (1945), report $n^{20}D$ 1.4705.

(8) P. Sabatier and A. Mailhe, *Ann. chim.*, [8] **10**, 527 (1907), report b.p. 181° (755 mm.) for this substance prepared in another manner.

(9) O. Wallach, *Ann.*, **359**, 287 (1908).

water. The filtered solution was extracted with benzene and then acidified with dilute sulfuric acid. The liberated cyclohexanecarboxylic acid was taken up in several portions of benzene, freed of solvent and warmed with 5 ml. of thionyl chloride. Treatment with 4 g. of aniline in benzene solution yielded the anilide, which was recrystallized from aqueous ethanol; yield 1.4 g., m.p. 147–148°.[10]

(10) A. M. Schwartz and J. R. Johnson, THIS JOURNAL, **53**, 1065 (1931), report m.p. 146° for cyclohexanecarboxanilide.

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Preliminary Investigations on the Preparation of Optically Active Peptides Using Mixed Carbonic–Carboxylic Acid Anhydrides

BY JAMES R. VAUGHAN, JR.

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The use of mixed carbonic–carboxylic acid anhydrides for the synthesis of peptides has been reported recently from this Laboratory[1] and independently from two European laboratories.[2] The general over-all equation for the reaction may be given by

$$\text{X-NHCH(R)COOCOOR''} + \text{H}_2\text{NCH(R')COOR''} \longrightarrow \text{X-NHCH(R)CONHCH(R')COOR''} + \text{R'''OH} + \text{CO}_2\uparrow$$

In our latest publication, the general nature of the reaction was demonstrated and it was reported that no racemization was observed in the preparation of simple, optically active dipeptide derivatives. The behavior of larger, optically active peptides, however, in which racemization may occur by mechanisms not operative in the case of dipeptides was not studied.

In the present work, the investigation of the retention of optical activity by this method of synthesis has been extended to a study of the reaction of amino acid esters with the mixed anhydrides of carbobenzoxy dipeptide acids in which the terminal amino acid having the free carboxylic function is optically active.

As model compounds, two dipeptide derivatives, carbobenzoxyglycyl-L-leucine and carbobenzoxyglycycl-L-phenylalanine, were examined. The first of these formed a toluene-soluble triethylamine salt and was caused to react with isobutylchlorocarbonate and then with methyl glycinate, by a modification of the method previously described,[1] to give methyl carbobenzoxyglycyl-L-leucylglycinate in 60% yield after purification. A 7% yield of the DL-isomer was also isolated.

The triethylamine salt of carbobenzoxyglycyl-L-phenylalanine, however, was only slightly soluble in toluene, and it was necessary to add a second solvent to effect solution. The use of toluene as the main solvent was desirable in order to obtain reaction temperatures of about −5° for the anhydride-forming step. When chloroform was used for this purpose and the reaction was carried through using isobutyl chlorocarbonate and ethyl glycinate in the usual manner, almost complete racemization occurred. Ethyl carbobenzoxyglycyl-DL-phenylalanylglycinate was obtained in 64% yield, whereas only 4% of the L-isomer was formed.

On further investigation, it was found that the amount of racemization observed could be greatly reduced by using the minimum amount of chloroform (1:8) necessary to solubilize the salt starting material and by reducing the time allowed for mixed anhydride formation to about 5 minutes. A summary of this work appears in the Experimental section.

When the use of chloroform in the anhydride-forming step was avoided and dioxane or tetrahydrofuran was used in its place, practically no racemization occurred. Thus, using a toluene–dioxane (5:2) solvent system in the above preparation, a 77% yield of ethyl carbobenzoxyglycyl-L-phenylalanylglycinate and only 2% of the DL-isomer was obtained after purification of all fractions. The use of dioxane alone as the solvent necessitated a slightly higher reaction temperature for the anhydride-forming step and resulted in a 64% yield of the L-isomer and 7% of the DL-form. The use of tetrahydrofuran alone, on the other hand, caused no detectable racemization and the pure L-isomer was isolated in 60% yield.[3]

As a check on the optical purity of ethyl carbobenzoxyglycyl-L-phenylalanylglycinate, the tripeptide was also prepared from a mixed isobutylcarbonate–carbobenzoxyglycine anhydride and ethyl L-phenylalanylglycinate. The product obtained was more difficult to purify than the one prepared from the carbobenzoxy dipeptide acid, but its optical rotation and melting point were in good agreement with those previously observed.

In connection with the above work, it was found that the over-all reaction time could be greatly shortened from that previously reported. Optimum time for anhydride formation at −5° is in the neighborhood of 5 to 10 minutes. However, this varies with the individual preparation. Also, after addition of an amino acid or peptide ester to a solution of the preformed mixed anhydride, the amide-forming reaction may be completed rapidly by heating the reaction mixture to reflux and then cooling.

Experimental[4]

Methyl Carbobenzoxyglycyl-L-leucylglycinate.—A solution of 3.22 g. (0.01 mole) of carbobenzoxyglycyl-L-leucine,[5] m.p. 99–100°, $[\alpha]^{25}\text{D}$ −9.5 ± 0.4° (*c* 5, ethanol), and 1.02 g. (0.01 mole) of triethylamine in 100 cc. of toluene was cooled to −5° and 1.37 g. (0.01 mole) of isobutyl chlorocarbonate added with stirring. After 10 minutes at this temperature, an 0.89-g. (0.01 mole) sample of methyl glycinate[6] was added with good stirring and the mixture was then heated rapidly to reflux and immediately cooled. Some of the product separated as a colorless oil. The reaction mixture, therefore, was stirred vigorously with 75 cc. of saturated sodium bicarbonate solution and the resulting heterogeneous mixture allowed to stand overnight at room temperature. The product separated from the toluene phase as colorless crystals, wt. 2.75 g. (70%), m.p. 131.5–132°. The material was recrystallized by dissolving it in

(1) J. R. Vaughan, Jr., THIS JOURNAL, **73**, 3547 (1951); J. R. Vaughan, Jr., and R. L. Osato, *ibid.*, **74**, 676 (1952).

(2) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).

(3) The yield before recrystallization was 83%, m. p. 112–115°.

(4) All melting points were taken on a Fisher–Johns block and are corrected.

(5) M. A. Stahmann, J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **164**, 759 (1946).

(6) M. Frankel and E. Katchalski, THIS JOURNAL, **64**, 2264 (1942).