these findings. Two different lots of synthetic tetrahydrocannabinol were prepared, and the rabbits used were shown to be responsive on subsequent days to fresh extracts of charas made with ethanol, evaporated, and taken up with acetone for testing.

We are indebted to Dr. C. E. Redemann for the analyses reported in this paper, and wish to thank him for many helpful suggestions made during the course of this work.

Summary

1. 3-*n*-Amylphenol has been prepared by two methods.

2. Derivatives of 6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyran substituted in the 3-position by *n*-amyl, methyl, hydroxy, butyloxy, butyroxy, ethoxy and acetoxy groups have been prepared.

3. Corresponding derivatives of 6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran have also been prepared.

4. Neither of these two series of pyrans exhibits any significant degree of marihuana activity in dogs or rabbits.

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[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS]

The Optical Configuration of Glutamic Acid Isolated from Casein Hydrolyzates by Six Procedures[1]

BY JEANETTE C. OPSAHL AND L. EARLE ARNOW[2]

Kögl, *et al.*,[3,4,5] have claimed that the modification of the Foreman procedure employed by Chibnall, *et al.*,[6] isolates preferentially *l*(+)-glutamic acid, leaving much of the *d,l*-glutamic acid in the mother liquor. For example, they[5] isolated 2.472 g. of *l*(+)-glutamic acid from 23.4 g. of pig kidney protein by Chibnall's procedure. Two grams of *d,l*-glutamic acid then was added to the mother liquor, and the isolation was repeated. 1.290 g. of glutamic acid was isolated; the sample was found to contain 0.9335 g. of *l*(+)-glutamic acid and 0.3569 g. of *d*(−)-glutamic acid. The interpretation of this type of experiment is complicated by the possibility that the original isolation might not have been quantitative. In other words, the material recovered in the second isolation conceivably might have reflected more or less accurately the composition of the glutamic acid present in the mother liquor. Chibnall and his collaborators[6] isolated small amounts of *d,l*-glutamic acid from both normal and malignant tissue protein hydrolyzates by their procedure. However, they did not report experiments in which *d,l*-glutamic acid had been added to the hydrolyzate prior to isolation.

Graff, Rittenberg and Foster[7] added *d,l*-glutamic acid to protein hydrolyzates, and found that the material isolated by their modified Foreman procedure contained both optical forms of glutamic acid. However, they were investigating the optical composition of the glutamic acid in the hydrolyzates by means of an isotope (N^{15}) dilution method, and the percentages of *d*(−)-glutamic acid in the material actually isolated were not given in their paper.

It has been shown in several laboratories[8,9,10] that glutamic acid slowly racemizes in boiling hydrochloric acid solutions. Several reports describing the isolation of glutamic acid containing small percentages of *d*-isomer have been recorded.[6,11,12,13] This latter finding casts some doubt on the accuracy of the isotope dilution method as employed by Graff, *et al.*[7] If the figure reported by these workers for the *d*-isomer content of the glutamic acid of tissue protein hydrolyzates (not more than 0.5 ± 0.5%) is accepted, it then becomes necessary to assume that the methods

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TABLE I

GLUTAMIC ACID HYDROCHLORIDE ISOLATED FROM CASEIN BY VARIOUS PROCEDURES

Method, with preliminary	Total crude yield, g.	Final yields, g.	C,[a] %	H,[a] %	N,[a] %	$[\alpha]_D$[b]	*d*-1somer, %	Calcd. final yields, %
1: clarification with Cu_2O	...	1.782	32.79	5.57	7.70	+28.4	5.1	46
2: pptn. as Ca salt	2.330	1.979	32.53	5.61	7.62	+29.9	2.7	51
3: pptn. as Ba salt	3.302[c]	1.715	[d]	[d]	7.58	+29.0	4.1	44
4: extn. with butanol	1.750	1.129	32.50	5.54	7.54	+30.0	2.5	29
5: clarification with ZnO	...	1.300	32.73	5.75	7.63	+27.7	6.2	34
6: sepn. as pyrrolidone-carboxylic acid	2.697	2.389	33.13	5.51	7.58	+29.9	2.7	62

[a] Theoretical: C, 32.71%; H, 5.49%; N, 7.63%. Carbon and hydrogen analyses were done by the Organic Microanalytical Laboratory, University of Minnesota. [b] Theoretical: +31.6°, calculated for free *l*(+)-glutamic acid in 9% hydrochloric acid. [c] Grossly contaminated with barium chloride. [d] This sample accidentally lost prior to carbon and hydrogen analyses.

employed by others preferentially concentrate the *d*-isomer. Since the *d,l*-form is about twice as soluble as the *l*-form in the hydrochloric acid solution usually used for crystallization,[4,7] it seems unlikely that such a preferential concentration occurs, at least in cases in which seeding is not employed.

Experimental Plan and Results

In the first group of experiments, glutamic acid was isolated from aliquots of a single casein hydrolyzate by means of the 6 different procedures listed in Table I. The results indicate that casein hydrolyzates contain small percentages of *d*(−)-glutamic acid. The figures recorded for the *d*-isomer contents of the isolated samples cannot be explained by assuming a large experimental error in the determinations of specific optical rotations. The figures obtained by us for the specific optical rotation of pure *l*(+)-glutamic acid (in 9% hydrochloric acid) have ranged from +31.0 to +32.5°. The average figure obtained in a large number of determinations was +31.6°.

The possibility that drying the casein at 110° might have caused some racemization of the combined glutamic acid was investigated. A sample of casein was heated at 110° for one week. The glutamic acid subsequently isolated by the pyrrolidone-carboxylic acid method was found to contain only 2.5% of *d*-isomer. This is essentially the same figure as that obtained after a much shorter period of drying (Table I).

In calculating the yields listed in Table I (last column), it has been assumed that the hydrolyzate prepared from 100 g. of thoroughly dried casein contains 21.77 g. of glutamic acid. This figure was reported by Foreman[14]; and figures approximating this have been obtained also with the butyl alcohol extraction method[15] and by the pyrrolidone-carboxylic acid method.[16]

In this Laboratory, the pyrrolidone-carboxylic acid method has given the highest yields of pure material. Moreover, the initial crude material obtained with this procedure contains smaller amounts of contaminants than is the case for any of the other methods used. Since the pyrrolidone-carboxylic acid procedure involves prolonged heating in aqueous solution at 100°, it might be supposed that some racemization would occur. However, numerous experiments with solutions of *l*(+)-glutamic acid have demonstrated that no detectable racemization occurs. It has been found also that samples isolated from glutamic acid solutions by this method reflect accurately the optical composition of the original racemic mixture. For example, a sample composed of 25 parts of *l*(+)-glutamic acid hydrochloride and 75 parts of *d,l*-glutamic acid hydrochloride was partly neutralized with sodium hydroxide until the resulting solution was green to brom cresol green (approximately, *p*H 5). At this *p*H, as Wilson and Cannan[17] have shown, the conversion to pyrrolidone-carboxylic acid is not complete. After boiling for fifty hours, extraction with ethyl acetate, and isolation of the hydrochloride, the material obtained consisted of 26% *l*(+)-glutamic acid hydrochloride and 74% *d,l*-glutamic acid hydrochloride. Therefore, even though conditions purposely were adjusted so that recovery was far from complete (yield, 37%), nevertheless, within experimental error, the recovered glutamic acid hydrochloride had the same optical composition as the original sample.

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TABLE II

GLUTAMIC ACID HYDROCHLORIDE ISOLATED FROM CASEIN + *d,l*-GLUTAMIC ACID BY VARIOUS PROCEDURES

Method[a]	Total crude yields, g.	Final yields, g.	Nitrogen (theory, 7.63%), %	$[\alpha]_D$[a]	*d*-1somer, %	Average *d*-1somer, %	Total final yields, g.	Calcd. final yields, %
1	5.184	2.896	7.60	+22.7	14.1	18.1	3.233	53
		0.337	7.67	− 1.4	52.2			
2	4.198	2.970	7.65	+23.9	12.2	16.4	3.311	54
		0.341	7.56	− 2.1	53.3			
3	4.107	1.593	7.69	+28.7	4.6	17.7	2.714	44
		0.572	7.69	+14.6	26.9			
		0.549	7.55	+ 2.3	46.3			
4	3.083	1.821	7.55	+22.6	14.2	15.4	1.959	32
		0.138	7.68	+11.5	31.8			
5	3.056	1.285	7.65	+24.0	12.0	10.7	1.638	27
	0.431[b]	0.353[b]	7.71	+27.9	5.9			
6	3.980	2.803	7.68	+24.1	11.9	18.8	3.510	57
		0.418	7.68	+ 6.1	40.3			
		0.289	7.60	− 3.0	54.8			

[a] See Table I. [b] Isolated from "insoluble fraction."

The data recorded in Table II were obtained by isolating glutamic acid hydrochloride from a casein hydrolyzate to which had been added a known amount of *d,l*-glutamic acid. Since 12 g. of dry *d,l*-glutamic acid was added to hydrolyzate equivalent to 95 g. of dry casein, the theoretical *d*-isomer content of the glutamic acid in the mixture was 18.4%. This calculation involves the assumption that no *d*-isomer was formed during the hydrolysis. If it is assumed that the hydrolysis caused a racemization of 10% of the glutamic acid originally present, the theoretical content of *d*-isomer becomes 21.5%. Foreman's figure for the glutamic acid content of casein has been used for these calculations.

It appears justifiable, therefore, to assume that the *d*-isomer content of the glutamic acid in the casein hydrolyzate was something between 18.4 and 21.5%. The data recorded in Table II indicate that the methods yielding samples most closely approximating the theoretical value were the pyrrolidone-carboxylic acid method and the cuprous oxide (Abderhalden–Fuchs) method. The two variations of the Foreman procedure used in this investigation (methods 2 and 3) yielded samples containing, respectively, 76–89% and 82–96% of the calculated theoretical *d*-isomer content. The least efficient method was the zinc oxide procedure, which yielded a sample containing only 50–58% of the calculated amount of *d*-isomer.

Chibnall and his collaborators[6] have claimed that the cuprous oxide method preferentially isolates *d,l*-glutamic acid; *i. e.*, that the first fraction crystallizing from the clarified hydrolyzate contains a higher percentage of *d*-isomer than do subsequent fractions. The data in Table II appear to indicate that the initial material isolated by this method does contain a higher percentage of *d*-isomer than do the first samples isolated by the Foreman or pyrrolidone-carboxylic procedures. However, in our experience, invariably it has been found that the first purified fractions obtained from hydrolyzates clarified with cuprous oxide contain less *d*(−)-glutamic acid than do fractions isolated from the original mother liquor. For example, the first fraction isolated from the hydrolyzate to which had been added *d,l*-glutamic acid contained 14.1% *d*-isomer. A crystalline fraction isolated subsequently from the mother liquor had a much higher *d*-isomer content (52.2%). Seeding has not been employed in our isolations.

It is possible that the type of results obtained with casein would not have been obtained if crude tissue protein had been used. In other words, substances absent from casein, but present in tissue, may influence the yield of *d*-isomer. We plan to investigate this possibility.

Experimental

Preparation of Hydrolyzates.—A sample of casein (Hoffman–LaRoche) was dried for thirty-six hours in an oven (105°). Ninety-five grams of the dry protein was mixed with a liter of 20% hydrochloric acid, and the mixture was boiled gently under reflux for twenty hours. After the removal of the majority of the hydrochloric acid

by several distillations under reduced pressure, the hydrolyzate was diluted to a volume of 200 cc. Thirty cc. aliquots then were used for each of the procedures summarized in Table I.

A second sample of the dry casein (95 g.) was treated as just described, except that 12 g. of dry *d,l*-glutamic acid was added to the hydrolyzate before final dilution to a volume of 200 cc. Thirty-cc. aliquots of this hydrolyzate were employed in obtaining the results listed in Table II.

The *d,l*-glutamic acid was prepared from *l*(+)-glutamic acid by the method of Arnow and Opsahl.[18]

Isolation of Glutamic Acid Hydrochloride. Method 1.—The modification of the Abderhalden–Fuchs procedure[19] employed by Kögl, Erxleben and Akkerman[4] was used, except that crystallization was allowed to proceed without seeding.

Method 2.—The method used was that of Chibnall, *et al.*[6] No attempt was made to isolate aspartic acid.

Method 3.—Graff, *et al.*[7,20] did not publish the details of their procedure. The procedure used by us is illustrated by the following description of the isolation of glutamic acid hydrochloride from the hydrolyzate to which had been added *d,l*-glutamic acid.

Thirty cc. of hydrolyzate was added to 100 cc. of water. A slight excess of barium hydroxide was added with stirring, and the volume of the alkaline solution was made to 185 cc.; 925 cc. of 95% alcohol was added with stirring, and the mixture was placed in the refrigerator for four days. The insoluble material then was filtered off, and dissolved in warm water (volume now 310 cc.); 1550 cc. of 95% alcohol was added with stirring, and the mixture allowed to remain in the refrigerator for three days. The insoluble barium salts were filtered off and dissolved in warm water containing sufficient hydrochloric acid to cause complete solution. Barium then was removed with sulfuric acid. The precipitated sulfate was washed several times with hot water, and the washings were combined with the amino acid solution. The combined solutions were evaporated to a small volume (approximately 20 cc.) under reduced pressure. This concentrated solution was saturated with dry hydrogen chloride gas at ice-bath temperature, and was stored at 0° for several days. The precipitated glutamic acid hydrochloride was filtered off (sintered glass filter); washed successively with cold concentrated hydrochloric acid, absolute alcohol, and ether; and dried in a desiccator over calcium chloride and potassium hydroxide. After its weight had been recorded, it was recrystallized from a minimum quantity of 20% hydrochloric acid. The other fractions listed in Table II were isolated from the mother liquor filtrates.

Method 4.—Thirty cc. of hydrolyzate was diluted with 10 cc. of water, and solid calcium hydroxide was added until the *p*H of the solution reached 6.1, as indicated by the glass electrode. This solution was extracted in a continuous extractor with butyl alcohol for seventy-four hours. The butanol-insoluble fraction was filtered (*p*H of solution now 8.0), and was freed of calcium with oxalic acid solution. The calcium-free solution was concentrated under reduced pressure to a volume of approximately 20 cc., and was saturated with cold hydrogen chloride gas at ice-bath temperature. After the solution had remained for several days at 0°, glutamic acid hydrochloride was isolated and purified in the usual way.

Method 5.—The procedure will be illustrated by a brief description of the isolation of the material recorded in Table II.

Thirty cc. of hydrolyzate and 20 g. of zinc oxide were added to 100 cc. of water. The mixture was heated to boiling for a few minutes, after which it was allowed to remain at 0° for one week. The insoluble material was filtered off, and washed with hot water. (This precipitate was saved for further investigation.) Zinc was removed from the filtrate and washings with hydrogen sulfide, and the filtrate was evaporated under reduced pressure to a volume of approximately 20 cc. After the addition of 5 cc. of concentrated hydrochloric acid, the solution was heated under reflux for two hours (to convert any pyrrolidone-carboxylic acid present to glutamic acid). After saturation of the solution with dry hydrogen chloride gas at ice-bath temperature, and storage of the concentrated hydrochloric acid solution in the refrigerator for several days, glutamic acid hydrochloride was isolated in the usual manner.

The original insoluble material (see above) was dissolved in 10% acetic acid. After removal of the zinc with hydrogen sulfide, it was evaporated to dryness under reduced pressure (to remove acetic acid). The residue was dissolved in 20 cc. of 9% hydrochloric acid; 5 cc. of concentrated hydrochloric acid was added; and the solution was heated under reflux for two hours. The small fraction of glutamic acid indicated in Table II was isolated and purified as already described.

Method 6.—This procedure was based on the suggestive experiments of Wilson and Cannan[17] and of Pucher and Vickery.[21] It will be illustrated by a brief description of the isolation of the material listed in Table II.

Thirty cc. of hydrolyzate was diluted with 30 cc. of water. The *p*H of this solution was adjusted to 3.3 (glass electrode) with 15 *N* sodium hydroxide. This neutralized solution was boiled gently under reflux for fifty hours (to convert glutamic acid to pyrrolidone-carboxylic acid). After concentration to a volume of about 30 cc., insoluble material was filtered off and washed with a small amount of cold water. The combined filtrate and washings were adjusted to *p*H 2.5 (glass electrode) with hydrochloric acid, after which pyrrolidone-carboxylic acid was removed by extracting with ethyl acetate for fifty hours. Further extraction for fifty hours did not increase the yield. Ethyl acetate was removed from the crude pyrrolidone-carboxylic acid by evaporation on a water-bath. The crude material was dissolved in 40 cc. of 9% hydrochloric acid, and the solution was heated under reflux for two hours (to convert pyrrolidone-carboxylic acid to glutamic acid hydrochloride). The condenser was removed, and heating was continued until the volume of the solution was approximately 20 cc. After saturation with hydrogen chloride gas at ice-bath temperature and storage at 0°, glutamic acid hydrochloride was isolated and purified.

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Nitrogen Analysis.—The nitrogen contents of the isolated samples were determined by the method of Cavett.[22] Each recorded nitrogen value represents the average of at least two independent determinations.

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Summary

1. Glutamic acid hydrochloride was isolated from hydrolyzed casein by six different procedures. The percentages of *d*-isomer in the isolated samples varied from 2.5 to 6.2%.

2. The isolations were repeated, using this time a casein hydrolyzate to which had been added a known amount of *d,l*-glutamic acid. The methods yielding samples having *d*-isomer contents closest to the theoretical content were the pyrrolidonecarboxylic acid procedure and the cuprous oxide procedure. Two modifications of the Foreman procedure yielded samples containing, respectively, 76–89 and 82–96% of the theoretical content of *d*-isomer. The poorest percentage yields of *d*-isomer were obtained with the zinc oxide procedure.

3. Methods for isolating glutamic acid by the pyrrolidone-carboxylic acid procedure and the zinc oxide clarification procedure, and modifications of the barium salt and butyl alcohol extraction procedures are described.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NEW YORK UNIVERSITY]

Products from the Wurtz Reaction and the Mechanism of their Formation[1]

BY ALFRED SAFFER AND T. W. DAVIS

The combination of methyl radicals with each other does not seem to occur under conditions where other combinations proceed readily.[2] The symmetrical approach of two methyl groups according to calculations of Kimball[3] and of Kassel[4] leads to a product of very short life. There is a possibility that methyl and ethyl radicals, being unlike, may combine more readily than like radicals, in which event the cross combination would result in a preponderant formation of propane, for example, in a Wurtz synthesis involving a methyl and an ethyl compound. The Wurtz reactions seem to offer a way to generate free radicals in any desired proportions so that one may study the relative probability of particular free radical combinations. The results of such a study are reported in this paper.

Von Hartel and Polanyi[5] found the reaction of methyl iodide with sodium vapor to proceed with zero energy of activation, and the reaction ought to go at every collision, therefore, regardless of the temperature. But below 300°, there is no measurable reaction because of the low vapor pressure of the metal. Above 300° with pressures of iodide in the neighborhood of 100 mm., the reaction with methyl or ethyl iodide proceeds at a convenient rate. Our experiments were conducted at 320° and in the absence of solvent because the compounds used as solvents in the ordinary Wurtz syntheses often enter into the reactions.[6] Consequently, in undertaking a study of mixed free radical reactions, we have found it convenient and desirable to depart markedly from the conventional details of the Wurtz synthesis.

Experimental

Method.—The apparatus for this study was used in the following way. About nine grams of sodium was placed in the large bulb of the addition tube, D, and the open end, E, was sealed off. The system was evacuated and the sodium melted by heating with a flame until it flowed into the lower and smaller bulb, where it was further melted and allowed to run into the reaction vessel, C.

During a run, the sodium was kept at 320° by means of the furnace, B. The reactant vapors were introduced by dropping liquid from the

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