We observed, however, that *l*-leucine and *l*-glutamic acid can be acetylated with ketene even under acid conditions and at reflux temperatures without causing complete racemization. These two amino acids can also be acetylated with acetic anhydride in aqueous acetic acid to give the active acetyl amino acids. These procedures appear practical for the preparation of acetyl-*l*-leucine and acetyl-*l*-glutamic acid since they eliminate a separation of salts from the acetylated amino acids and give yields of about 50% of the pure acetylated compounds. The purity of the compounds was proved by de-acetylating them with dilute hydrochloric acid to the original amino acids of unchanged rotation. This method cannot be considered generally applicable to all active amino acids since it was found that *l*-arginine racemizes completely and *l*-cystine decomposes as observed previously (3) when treated with ketene in water solution.

Experimental

N-Acetyl-l-leucine.—Pure *l*-leucine was prepared by recrystallization of its nasylate[4] until it had a constant melting point of 189–191° and a constant rotation of $[\alpha]^{25}D$ +12.84 (4% in ethanol). On decomposing the leucine nasylate in ethanol solution with ethanolamine, *l*-leucine was obtained which was recrystallized from water to a constant rotation of $[\alpha]^{25}D$ +14.7 (9% solution in 4.5 *N* hydrochloric acid); reported $[\alpha]^{24}D$ +15.33 (4), $[\alpha]^{27}D$ +16.5 in 20% HCl (2), $[\alpha]^{25}D$ +13.91 (9.075% in 4.5 *N* HCl).[5] A stream of ketene (0.44 mole/hr.) was passed through a solution of 19 g. of *l*-leucine in 1 l. of water until a Van Slyke determination showed practically no amino nitrogen (about 9 hours were required). The solution reached a temperature of 40–50° during the passage of the ketene. The *p*H of the final solution was 3.0, and titration showed the solution to have an acidity equal to 4 *N* acetic acid. After carbon treatment the solution was concentrated *in vacuo* with repeated additions of water to drive off acetic acid. A white precipitate was obtained, weighing 16 g. after washing and drying. This material on recrystallization from ethanol yielded 10.6 g. (44% of theoretical) of acetyl-*l*-leucine, m.p. 183–184°, $[\alpha]^{25}D$ −23.3 (3% in ethanol); reported $[\alpha]^{29}D$ −21.0 (3% in ethanol)[2] and $[\alpha]D^{29}$ −16.99.[6]

Anal. Calcd. for $C_8H_{15}O_3N$: N, 8.1; neut. equiv., 173.2. Found: N, 8.1; neut. equiv., 174.8.

Passing ketene into a water solution of *l*-leucine under reflux gave similar results. Hydrolysis with 5 *N* hydrochloric acid gave *l*-leucine hydrochloride from which *l*-leucine was obtained in 70% yield $[\alpha]^{25}D$ +14.8. Addition of 100 g. of acetic anhydride to 19 g. of *l*-leucine in 500 g. of 40% acetic acid with stirring at 60° and isolating as described above gave 16.2 g. (65% yield) of acetyl-*l*-leucine, $[\alpha]^{25}D$ −22.9. Repeating the acetylation as described above in the absence of water gave racemized acetylleucine.

N-Acetyl-l-glutamic Acid.—*l*-Glutamic acid, $[\alpha]^{25}D$ +31.6 (5% solution in 10% hydrochloric acid) was treated in aqueous solution with ketene as described for *l*-leucine. N-Acetyl-*l*-glutamic acid was isolated in 47% yield, m.p. 194–195°, $[\alpha]^{25}D$ −15.7 (3% in water), $[\alpha]^{25}D$ +3.97 (2% in 1 *N* sodium hydroxide); reported m.p. 198–199°,[1,2] 195°,[7] 195–197°[8] and $[\alpha]^{24.5}D$ +3.9 resp. 3.83 (2% solution in 1 *N* NaOH)[2,3] and $[\alpha]D$ −22.7 (in water).[8]

Anal. Calcd. for $C_7H_{11}O_5N$: N, 7.4; neut. equiv., 94.6. Found: N, 7.3; neut. equiv., 95.5.

Hydrolysis with 5 *N* hydrochloric acid gave 80% yield of *l*-glutamic acid $[\alpha]^{25}D$ +31.7 (5% solution in 10% hydrochloric acid). Acetylating 20 g. of *l*-glutamic acid in 500 ml. of 40% acetic acid with 100 ml. of acetic anhydride gave 14 g. (55%) of acetyl-*l*-glutamic acid of $[\alpha]^{25}D$ −15.4 (3% in water).

(4) Bergmann and Stein, *J. Biol. Chem.*, **129**, 609 (1939).

(5) Dunn and Rockland, *Advances in Protein Chem.*, **III**, 354 (1947).

(6) Cherbuliez, Plattner and Ariel, *Helv. Chim. Acta*, **13**, 1390 (1930).

(7) Karrer, Escher and Widmer, *Helv. Chim. Acta*, **9**, 301 (1926).

(8) Knoop and Oesterlin, *Hoppe-Seylers Z.*, **170**, 186 (1927).

l-Arginine.—A solution of *l*-arginine in water was completely racemized after a stream of ketene was passed through the solution for several hours.

l-Cystine.—On passing ketene through a water solution of *l*-cystine a fine precipitate of sulfur was obtained.

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RECEIVED MARCH 10, 1950

The Preparation of Some Dialkylaminoalkylaldehydes

BY MILDRED YANDIK AND A. A. LARSEN

In the course of investigating compounds for analgesic activity two aminoaldehydes were prepared for screening. These compounds, 4-dimethylamino-2,2-diphenyl entanal and 4-dimethylamino-2,2-diphenyl-3-methylbutanal were obtained from the corresponding nitriles[1] by reduction with lithium aluminum hydride.[2] Isolation was somewhat complicated by the presence of the basic amino group in the molecule and in addition by the fact that distillation was of no benefit since the nitrile and aldehyde distill at the same temperature under reduced pressure. The presence of the quaternary carbon atom adjacent to the carbonyl group does not allow for the preparation of the more common aldehyde derivatives. Catalytic hydrogenation of the aldehydes resulted in the uptake of one mole of hydrogen and isolation of the corresponding alcohols.

Bockmühl[3] reported that 2,2-diphenyl-4-piperidinobutanal, prepared by Rosenmund reduction of the acid chloride hydrochloride, has about one-third the analgesic activity of methadone. Tests performed in this Laboratory under the direction of Dr. J. R. Lewis showed that 4-dimethylamino-2,2-diphenylpentanal has about one-fifth the activity of methadone, whereas 4-dimethylamino-2,2-diphenyl 3-methylbutanal is less active as an analgesic.

Experimental

4-Dimethylamino-2,2-diphenylpentanal Hydrochloride.—To a solution of 139 g. (0.5 mole) of 4-dimethylamino-2,2-diphenylbutanenitrile in 90 ml. of dry ether was added portionwise with stirring 5.5 g. (0.145 mole) of lithium aluminum hydride. After addition was complete the mixture was refluxed for four hours and left to stand overnight. After the addition of water, the gelatinous solid was removed by filtration and the ether layer extracted with dilute hydrochloric acid. The acid extract was washed with ether, made ammonical with concentrated ammonia and the free base extracted with ether. After drying, the ether solution was treated with alcoholic hydrogen chloride and the resultant oily solid was recrystallized from acetone–methanol to give 47 g. of aldehyde hydrochloride monohydrate, m.p. 122–124°. When dried in the vacuum oven at 100° for 96 hours the anhydrous hydrochloride was obtained, m.p. 187–189°.

Anal. Calcd. for $C_{19}H_{23}NO{\cdot}HCl$: C, 71.81; H, 7.61; N, 4.40; Cl, 11.15. Found: C, 71.74; H, 7.37; N, 4.41; Cl, 10.93.

Low pressure catalytic hydrogenation using platinum oxide, 1 g. of catalyst to 10 g. of aldehyde, gave 4-dimethylamino-2,2-diphenylpentanol hydrochloride,[4] m.p. 214–216°.

(1) E. M. Schultz, C. M. Robb and J. M. Sprague, THIS JOURNAL, **69**, 2456 (1947).

(2) L. Friedman, Abstracts 116th Meeting American Chemical Society, Atlantic City, N. J., 1949, p. 5M.

(3) M. Bockmühl and G. Ehrhart, *Ann.*, **561**, 75 (1948).

(4) M. E. Speeter, W. M. Byrd, L. C. Cheney and S. B. Binkley, THIS JOURNAL, **71**, 57 (1949).

4-Dimethylamino-2,2-diphenyl-3-methylbutanal Hydrochloride.—The reduction of 4-dimethylamino-2,2-diphenyl-3-methylbutanenitrile, 27.8 g. (0.1 mole), with lithium aluminum hydride in the above manner gave 7.5 g. of aminoaldehyde hydrochloride which was recrystallized from acetone–methanol, m.p. 187.8–192°.

Anal. Calcd. for: $C_{19}H_{23}NO \cdot HCl$: C, 71.81; H, 7.61; N, 4.40. Found: C, 71.75; H, 7.34; N, 4.47.

Catalytic reduction as indicated above gave 4-dimethylamino-2,2-diphenyl-3-methylbutanol hydrochloride, m.p. 200–201°.

Anal. Calcd. for $C_{19}H_{25}NO \cdot HCl$: C, 71.34; H, 8.19; Cl, 11.08. Found: C, 71.58; H, 8.39; Cl, 11.00.

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COMMUNICATIONS TO THE EDITOR

PYRIDOXAL PHOSPHATE, THE COENZYME OF THIOETHER-CLEAVAGE

Sir:

In a previous report[1] the activation of certain preparations of the enzyme responsible for the cleavage of thioethers (*e.g.*, cystathionine with the formation of cysteine) by relatively large amounts of folic acid was described. Since that time, it has been found that derivatives of folic acid (conjugates and citrovorum factor) were without effect. The failure of these derivatives to activate the preparations and the limited results obtained in further studies with folic acid led us to consider other possibilities as to the identity of the dialyzable component. It has been found that minute amounts of pyridoxal phosphate[2] activated all preparations of the enzyme—fresh, aged or dialyzed. Maximal activation was obtained with 0.5 γ of pyridoxal phosphate per ml. of digest. Djenkolic acid,[3] 10 mg., and 1 ml. enzyme[4] in a total volume of 10 ml. 0.1 *M* sodium citrate were incubated for 30 minutes at 37° with amounts of pyridoxal phosphate varying from 0.1 to 10 γ per ml. With the fresh enzyme, maximal activity, 1.1 mg. of cysteine was obtained with 0.5 γ of pyridoxal phosphate; the control was 0.5 mg. of cysteine. After dialysis overnight against acetate buffer, 0.1 *M*, *p*H 5.5, the activity was reduced to 0.2 mg. of cysteine and was restored to 1.0 mg. of cysteine upon the addition of 0.5 γ of pyridoxal phosphate per ml. of digest. These amounts of pyridoxal phosphate are of the same order of magnitude as required for the transamination and decarboxylation enzymes and are compatible with the amounts predicted from the absorption spectrum of the enzyme.[1] It would appear, therefore, that pyridoxal phosphate is the coenzyme of the cleavage-enzyme.

When 10 mg. of pyridoxin and 50 mg. of adenosinetriphosphate were incubated in 10 ml. of saline with 1 ml. of homogenate of liver tissue (1 g. in 10 ml.) for 15 minutes, an apparent content of 5.5 γ of pyridoxal phosphate per ml. (activation of dialyzed enzyme) was found. The addition of folic acid was found to increase markedly the amount of coenzyme formed. It would appear probable, therefore, that the effects of folic acid and of adenosinetriphosphate[5] on the cleavage will be found to be concerned with the synthesis of pyridoxal phosphate or a closely related compound. It is of interest that the ultraviolet absorption of the purified enzyme[1] may be interpreted as that of protein and pyridoxal phosphate.[6]

These and related studies will be reported in detail in the near future.

(1) F. Binkley, THIS JOURNAL, **72**, 2809 (1950).
(2) Obtained from Dr. W. W. Umbreit.
(3) M. D. Armstrong and V. du Vigneaud, *J. Biol. Chem.*, **168**, 373 (1947). Djenkolic acid is an easily prepared substrate.
(4) F. Binkley and D. Okeson, *J. Biol. Chem.*, **182**, 273 (1950).
(5) F. Binkley, *J. Biol. Chem.*, **155**, 39 (1944).
(6) W. W. Umbreit, D. J. O'Kane and I. C. Gunsalus, *ibid.*, **176**, 629 (1948).

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RECEIVED APRIL 30, 1951

"CITROVORUM FACTOR" ACTIVITY OF TETRAHYDROPTEROYLGLUTAMIC ACID

Sir:

The preparation of leucovorin (I),[1] 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid,[2,3] led to speculation as to its possible role in the transfer of "single-carbon fragments," following the suggestion which has been made for folic acid in such biological mechanisms.[4] It seemed feasible that I might be reversibly transformed to tetrahydropteroylglutamic acid (II) *in vivo* during such a process in which case II should have biological properties similar to those of I. II was synthesized by hydrogenation of 14.6 mg. of pteroylglutamic acid in 10 cc. of glacial acetic acid at room temperature, using 15 mg. of platinum oxide catalyst and a standard Ogg–Cooper micro-hydrogenation apparatus.[5] After 5.75 hours, reduction was complete; hydrogen uptake was 92.5% of the theoretical 2 moles. Subsequent operations were carried out under nitrogen to prevent oxidation. The catalyst was separated from the colorless solution of II by centrifugation, then aliquots were transferred to small test-tubes and vacuum-dried to a

(1) J. A. Brockman, Jr., *et al.*, THIS JOURNAL, **72**, 4325 (1950).
(2) E. H. Flynn, *et al.*, Abstracts of Papers, Am. Chem. Soc., 119th meeting, 18M (1951).
(3) B. Roth, *et al.*, in preparation.
(4) M. Gordon, *et al.*, THIS JOURNAL, **70**, 878 (1948).
(5) B. L. O'Dell, *et al.*, *ibid.*, **69**, 250 (1947).