chicine series[2] although other workers have noted the change in specific rotation of colchicine and desmethylcolchicine with solvent and concentration.[3,4]

Experimental

The specific rotation[5] of a sample of isocolchicine (m.p. 219–221°; calcd. for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31. Found: C, 65.94; H, 6.38), taken five minutes after preparation of a solution in pure chloroform (*c* 0.997), was −319°. This value decreased numerically with time as shown in Table I. With solutions of isocolchicine in chloroform containing 0.5–2.0% alcohol, a similar effect was observed but *not* with solution in alcohol nor with alcohol containing up to 0.5% chloroform. Colchicine did not show this behavior. This is in agreement with the findings of Bellet.[4]

A solution which had reached an equilibrium value (Sample 2, Table I) was evaporated to dryness on the steam-bath at atmospheric pressure. The residue was reconstituted in chloroform and the rotation was redetermined, $[\alpha]_D$ −260°. When the residue was dried for seven hours at 100° *in vacuo*, or heated with alcohol and water and then dried *in vacuo*, the rotation returned to −305°. However, when the residue was dissolved in ethyl acetate and evaporated to dryness three times,[6] the rotation of the reconstituted chloroform solution rose to the original value and again decreased numerically to the equilibrium value after standing 4.5 hours (sample 4, Table I). A plot of the log % change in specific rotation against time, using the average values given in Table I, is a straight line (Fig. 1).

These data suggest that the change is due to the formation of a stable complex between isocolchicine and chloroform. The nature of this complex is under investigation elsewhere.[3]

The infrared spectrum (*c* 10 mg./cc. in chloroform) exhibited no characteristics which could be associated with changes in the specific rotation of isocolchicine samples which had stood for 0, 1, 2 and 5 hours after solution in chloroform.

TABLE I

SPECIFIC ROTATION OF ISOCOLCHICINE IN CHLOROFORM

Sample →	1	2	3	Ave.	4
c →	0.997	1.000	1.000		1.000
t, min.	$[\alpha]^{22}_D$				
0[a]	−319°	−317°	−321°	−319°	−322°
15	310	308	...	309	...
30	300	...	298	299	...
60	286	281	288	285	283
120	269	265	273	269	266
240	260	254	262	259	259
360	259	253	260	257	...

[a] Initial readings were taken within the first five minutes after solution was effected.

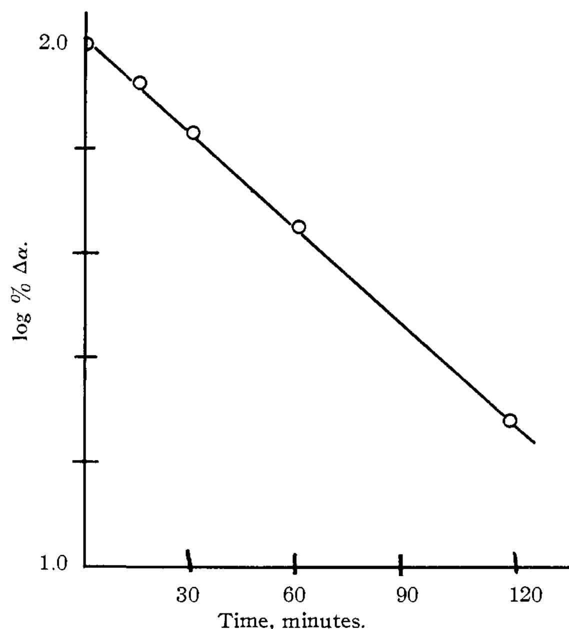

Fig. 1.—Change in specific rotation of isocolchicine with time.

(2) In a personal communication Dr. Henry Rapoport has informed us that he has observed similar variations in the specific rotation of a number of isocolchicine derivatives and will report his findings in greater detail at a later date.

(3) P. Bellet and P. Regnier, *Ann. pharm. franç.*, **10**, 340 (1952).

(4) R. M. Horowitz and G. E. Ullyot, unpublished data from these laboratories.

(5) Readings were taken on a Rudolph Precision Polarimeter in a 1-dm. tube at 24–26° with an estimated error of ±1.5°.

(6) The use of ethyl acetate was suggested by Dr. Rapoport.

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The Preparation of L-, D- and DL-Kynurenine[1]

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The purpose of this communication is to present a convenient procedure for the preparation of kynurenine from N-acetyltryptophan. The method is based on the ozonolysis procedure outlined by Witkop,[2] who used free DL-tryptophan. Protection of the amino group by acetylation prevents the extensive decomposition which otherwise occurs and permits the isolation of an initially purer and more efficiently recrystallizable kynurenine sulfate. The method has been found preferable to other procedures which we have tested. Isolation of kynurenine from the urine of rabbits fed tryptophan affords relatively low yields of a product difficult to purify.[3,4] The synthesis of kynurenine from *o*-nitrophenacyl bromide by Dalgliesh's modification[5] of the method of Butenandt and others,[6] yields an excellent product, but the method becomes highly involved if the starting material must also be prepared. The procedure outlined is adaptable to the preparation of L-, D- or DL-kynurenine in good yield from the corresponding isomer of acetyltryptophan.

Experimental

N-Acetyl-L- and N-acetyl-D-tryptophan were obtained by resolution of the DL-form with brucine.[7] Access of moisture to the solvent used was avoided. From anhydrous ethanol the brucine salt of N-acetyl-D-tryptophan separated in rosettes, $[\alpha]^{25}_D$ −18.0°, *c* 1% in water; from anhydrous methanol its diastereoisomer crystallized in rectangular prisms, $[\alpha]^{25}_D$ +0.5°. Removal of the brucine yielded N-acetyltryptophans which showed $[\alpha]^{25}_D$ −29.0° and $[\alpha]^{25}_D$ +30.1°, *c* 1% in water +1 equivalent of NaOH, in good agreement with recorded values.[8,9]

(1) From a thesis submitted by J. L. Warnell in partial fulfillment of the requirements for the degree of Master of Science in the Graduate College of the State University of Iowa.

(2) B. Witkop and G. Graser, *Ann. Chem.*, **556**, 103 (1944).

(3) Y. Kotake and J. Iwao, *Z. physiol. Chem.*, **195**, 139 (1931).

(4) R. E. Kallio and C. P. Berg, *J. Biol. Chem.*, **181**, 333 (1949).

(5) C. E. Dalgliesh, *J. Chem. Soc.*, 137 (1952).

(6) A. Butenandt, W. Weidel, R. Weichert and W. von Derjugin, *Z. physiol. Chem.*, **279**, 27 (1943).

(7) A. C. Shabica and M. Tishler, THIS JOURNAL, **71**, 3251 (1949). The N-acetyl-DL-tryptophan used was kindly provided by The Dow Chemical Company.

(8) V. du Vigneaud and R. R. Sealock, *J. Biol. Chem.*, **96**, 511 (1932).

(9) C. P. Berg, *ibid.*, **100**, 79 (1933).

Ozonolysis was accomplished by bubbling ozone in four fine streams into a suspension of 15 g. (0.061 mole) of finely powdered N-acetyltryptophan in 500 ml. of glacial acetic acid in a reaction cylinder 30 cm. high. The reaction mixture was maintained at a temperature of 20°, and was stirred magnetically. The ozone generator was similar to one described in the literature,[10] but contained no inner cooling jacket, the outer water-bath being kept at 10°. The electrodes were of copper wire and all non-glass connections were made with Tygon tubing. Calibration[10] showed that, under the conditions maintained, the ozonizer produced 60 l. of 2.3% ozone (0.061 mole) from oxygen at a potential of 10,000 volts in one hour. This was essentially quantitatively absorbed by the acetyltryptophan, as judged by the very slow release of iodine in the attached 5% KI trap during the reaction. The endpoint of the ozonolysis was evidenced by the accelerated liberation of iodine. The DL modification of N-acetyltryptophan fails to dissolve completely in the glacial acetic acid until the approach of the end-point of the reaction, marked in this instance also by clearance of the opalescence. Continued passage of ozone in excess of one equivalent (0.061 mole) lowers markedly the quality of the kynurenine sulfate initially obtained.

Hydrolysis and Isolation.—Hydrolysis was most readily accomplished by adding 60 ml. of 37% HCl to the reaction mixture and heating on the steam-bath under a reflux condenser fitted with a bunsen valve. During the 5.5 hours found necessary, 3 additions, 20 ml. each, of 37% HCl were made. Subsequently 3.3 ml. of concd. H_2SO_4 (0.061 mole) were added and the mixture was concentrated under reduced pressure in an atmosphere of nitrogen to a thick sirup, which was dissolved in 250 ml. of hot 50% ethanol and partially decolorized with charcoal. The filtrate and washings were concentrated *in vacuo* to about 20 ml. and 3 volumes of hot ethanol were added. On cooling, 14.5 g. of buff needles of kynurenine sulfate separated (yield 77%). The sulfate recrystallized readily from 66% ethanol, after clarification with charcoal, in long, almost white needles. The presence of a slight excess of sulfuric acid greatly favors the crystallization.

The hydrolysis step can also be effected by concentrating the glacial acetic acid reaction mixture to 50 ml., adding 300 ml. of 2 *N* HCl and 3.3 ml. (0.061 mole) of concd. H_2SO_4 and heating on the steam-bath for 2 hours, but the kynurenine sulfate isolated when this procedure is followed is contaminated with a dark gritty coprecipitate which makes purification by recrystallization much more difficult.

When L-, D- or DL-kynurenine sulfate is air-dried at 40° it contains one molecule of water which can be removed by drying *in vacuo* at 100°. The anhydrous product is hygroscopic and gradually takes up water to re-form the monohydrate. Analyses of the D- and L-forms agree well with the following analyses of the DL modifications.

Anal. Calcd. for $C_{10}H_{12}N_2O_3 \cdot H_2SO_4 \cdot H_2O$: C, 37.03; H, 4.97; N, 8.64; S, 9.89. Found: C, 37.01; H, 5.13; N, 8.66; S, 10.2. Calcd. for $C_{10}H_{12}N_2O_3 \cdot H_2SO_4$: C, 39.21; H, 4.61; N, 9.15; S, 10.47. Found: C, 39.12; H, 4.84; N, 9.15; S, 10.65.

DL-Kynurenine was obtained by adding 2 equivalents of 4 *N* NaOH to a hot aqueous solution of the sulfate and filtering off the buff colored platelets which form on standing in essentially quantitative yield. D- and L-kynurenine are more soluble, hence were more easily obtained by the exact removal of the sulfate as $BaSO_4$ and concentration of the aqueous solution under reduced pressure in an atmosphere of nitrogen, until crystallization began, followed by the addition of 2 volumes of hot ethanol. The buff platelets may be purified by recrystallization from a minimal volume of hot 66% ethanol after clarification with charcoal. Following is the analysis of DL-kynurenine. Analyses of the D- and L-forms were in good agreement with it.

Anal. Calcd. for $C_{10}H_{12}N_2O_3$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.75; H, 6.00; N, 13.52.

Physical Properties of the Compounds Prepared.—The specific rotations and decomposition points of the optical modifications of kynurenine sulfate and kynurenine are summarized in Table I.

TABLE I

Optical form	$[\alpha]^{25}D$ c 1% in H_2O	M.p., °C., with dec.
Kynurenine sulfate, monohydrate		
L	+9.6°	178
D	−9.5	178
DL	0.0	173
Kynurenine sulfate, anhydrous		
L	+10.2°	178
D	−10.1	178
DL	0.0	173
Kynurenine		
L	−30.5°	191
D	+30.0	191
DL	0.0	218

Literature values for the specific rotation of kynurenine sulfate range from 8.5 to 10.7[3,4,11-13] and for kynurenine from 28.5 to 31.5.[3,6,11-13] The tendency of the sulfate to bind water is possibly responsible for the differences in its recorded physical constants. The decomposition points recorded in Table I were obtained in capillary tubes, heated 2° per min. from 20° below the m.p.

The ultraviolet absorption curves of the L-, DL- and D-forms of kynurenine sulfate were identical with the curve recorded by Dannenberg.[14]

Infrared absorption curves obtained with kynurenine sulfate monohydrate in Nujol paste showed broad absorption around 3.5 μ, sharp peaks at 5.74 and 5.90 μ, and a broad band with peaks at 6.28, 6.57 and 6.68 μ. Kynurenine in Nujol showed broad absorption around 3.4 μ and sharp peaks at 6.03 μ and at 6.20 and 6.35 μ. On paper chromatograms the L-kynurenine preparations migrated somewhat more rapidly than the D, and the DL produced two spots.[12]

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(10) L. I. Smith, F. L. Greenwood and O. Hudrlik, *Org. Syntheses*, **26**, 63 (1946).

(11) A. Butenandt and R. Weichert, *Z. physiol. Chem.*, **281**, 122 (1944).

(12) M. Mason and C. P. Berg, *J. Biol. Chem.*, **195**, 515 (1952).

(13) Y. Kotake, Jr., and N. Ito, *J. Biochem., Japan*, **25**, 71 (1937).

(14) H. Dannenberg, *Z. Naturforsch.*, **4b**, 327 (1949).