were easily separated from the dibasic acids by extracting the former with petroleum ether. From 200 mg. of diynoic acid there was isolated 30 mg. (23%) of pure azelaic acid, m.p. and m.m.p. 104–105°. Titration of the monobasic acid fraction with standard alkali indicated the presence of 0.243 mmole of acid. The *p*-bromophenacyl derivative of this acid melted at 67–69°; mixed with *p*-bromophenacyl caproate (m.p. 70–71°), the material showed m.p. 69°.

Attempted Preparation of Octadecadiyne-9,12-oic Acid from 1-Chlorohexadecadiyne-7,11.—A mixture of 23.7 g. (0.094 mole) of 1-chlorohexadecadiyne-7,11, 19 g. (0.12 mole) of diethyl malonate, 0.108 gram atom of sodium, 2 g. of potassium iodide and 300 ml. of absolute ethanol was boiled under nitrogen for 15 hours. (In a separate experiment it was determined that 93% of the theoretical amount of sodium ethoxide was consumed after 18 hours). The isolation procedure was the same as that described for the reaction with iodohexadecadiyne. Decarboxylation of the crude malonic acid in portions afford a total of 6.2 g. of monocarboxylic acid which on redistillation furnished 5.2 g. of product, m.p. 32–33°.

Anal. Calcd. for $C_{18}H_{28}O_2$: C, 78.2; H, 10.2. Found: C, 78.0; H, 10.2.

Quantitative hydrogenation of 0.216 g. of this material over palladium-on-barium sulfate catalyst in alcohol solvent indicated an absorption of 0.00308 mole of hydrogen. The value calculated for two triple bonds is 0.00312 mole. Two low-temperature crystallizations of the hydrogenation product afforded 0.20 g. (91%) of stearic acid, melting alone or admixed with an authentic sample at 70–71°.

Ozonolysis according to the procedure described for the ozonolysis of octadecadiyn-9,12-oic acid led to a mixture of dibasic acids, m.p. 110–128°, and a *p*-bromophenacylate of the monobasic acids melting at 40–62°. Isolation of a pure compound from either fraction failed.

In contrast to the 42–43° diynoic acid, the 32–33° acid could not be crystallized satisfactorily from methanol or from acetone. On contact with air the material immediately acquired a purple color. No linoleic acid tetrabromide could be isolated after adding bromine to the half-hydrogenation product of this low-melting acid.

Hydrogenation Experiments with Octadecadiyn-9,12-oic Acid (m.p. 42–43°).—The different effect of conditions on the relative rates of the first and second stages of hydrogenation is illustrated by the following experiments:

A.—When the volume of hydrogen absorbed in the hydrogenation of 96 mg. of octadecadiyn-9,12-oic acid (0.348 mmole) in 20 ml. of ethanol in the presence of 10 mg. of 5% palladium-on-strontium carbonate is plotted against time, a curve is obtained which is linear (after an induction period) during absorption of the first two moles of hydrogen. Close to the point corresponding to half-hydrogenation the slope changes sharply, and a second straight line is observed. From the graph the rates of hydrogen absorption in the first and second stages of the process were found to be 0.8 ml./min. and 0.025 ml./min., respectively.

B.—When the conditions were changed so that 495 mg. of octadecadiyn-9,12-oic acid (1.8 mmole) in 45 ml. of ethanol was hydrogenated over 45 mg. of catalyst, the hydrogenation curve was again represented by two intersecting lines. However, whereas the rate of hydrogen absorption in the first stage was 3 ml./min.—corresponding to a 3.75-fold change from the first experiment—the rate of hydrogen absorption in the second stage was 0.7 ml./min., corresponding to a 28-fold change.

Linoleic Acid Tetrabromide from Octadecadiyne-9,12-oic Acid (IV).—Hydrogenation of the diynoic acid, m.p. 42–43°, in ethanol over a 5% palladium-on-strontium carbonate catalyst until two moles of hydrogen were absorbed led to an oily product which boiled under a pressure of 0.005 mm. at an external temperature of 160–180°. Bromination of this material in petroleum ether permitted isolation of 35 mg. of white material (m.p. 108–112°), which after two crystallizations from ethanol melted at 113–114°. Mixed with an authentic sample of linoleic acid tetrabromide (m.p. 114–115°), the compound melted at 113–114°.

BOSTON, MASSACHUSETTS RECEIVED FEBRUARY 1, 1951[26]

(26) A summary of the material in the paper was originally submitted for publication (Oct. 13, 1950) as a Communication to the Editor.

[CONTRIBUTION FROM THE FURMAN CHEMICAL LABORATORY, VANDERBILT UNIVERSITY]

The Resolution of Amino Acids. III. Methionine[1]

BY GLYNN P. WHEELER[2] AND A. W. INGERSOLL

Methods based on the use of acetyl derivatives[3,4] and α-fenchylamines have been extended with fair success to the resolution of methionine into both active forms. A much more rapid and convenient resolution giving L-methionine has been effected through the salt of (+)-α-bromocamphor-π-sulfonic acid.

The original method of Windus and Marvel[5] using the N-formyl derivative and brucine, with some later modifications,[6,7] remains the only recorded chemical method for resolving methionine. Numerous biochemical methods have been employed recently, including the use of amino acid oxidases,[8,9] yeast fermentation,[10] the papain anilide synthesis[11] and the apparently more convenient selective enzymatic hydrolyses of various N-acyl derivatives[12] and esters.[13,14]

We now report that DL-methionine can be resolved fairly conveniently through the N-acetyl derivatives. N-Acetyl-D- and L-methionines were obtained in 82 and 40% yields, respectively, by successive use of (−)- and (+)-α-fenchylamine[15] and converted to the corresponding amino acids. The method is perhaps as reliable and convenient as that of Windus and Marvel and somewhat more adaptable to large scale use. However, since the racemic and active acetyl derivatives and salts are rather soluble, the procedures at each stage are considerably less convenient and give lower yields than were obtained in the analogous resolu-

(1) Taken from the Ph.D. thesis of Glynn P. Wheeler, September, 1950.

(2) National Institutes of Health Predoctorate Fellow, 1948–1950.

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tions of phenylalanine,[3] valine[3] and isoleucine.[4] A partial resolution also was effected with brucine, but α-phenylethylamine, cinchonine and quinine were not useful.

The resolution of monoamino acids directly through the salts of optically active sulfonic acids has occasionally been successful[16]; in most instances the salts concerned are too soluble. An attempted resolution of methionine by means of (+)-α-bromocamphor-π-sulfonic acid was abandoned by Vogler and Hunzicker[17] because the salts were regarded as too soluble and difficult to crystallize. We found, however, that substantially pure L-methionine bromocamphorsulfonate crystallizes promptly in 85% yield from a molar solution containing DL-methionine hydrochloride and ammonium bromocamphorsulfonate in equivalent amounts. Addition of aqueous ammonia regenerated pure L-methionine from the crystalline salt and D-methionine of 80–85% purity from the original filtrate. Ammonium bromocamphorsulfonate suitable for re-use was readily recovered. A modification using one-half equivalent of the ammonium salt was also successful. These procedures provide pure L-methionine with rather unusual ease and economy. D-Methionine was prepared similarly by use of ammonium (−)-α-bromocamphor-π-sulfonate. This agent[18] is too rare for general use but otherwise satisfactory. Attempted resolution of ammonium *dl*-α-bromocamphor-π-sulfonate with L-methionine hydrochloride failed.

Experimental

All melting points were taken with a calibrated A.S.T.M. thermometer without further correction. Solubility values are the average of duplicate determinations expressed as grams per 100 cc. of solution at 25 ± 0.5°. The values are moderately precise, sufficient for comparison and selection of solvents. Rotations were taken in 2-dm. tubes and are reproducible within ±0.2 to 0.3°. DL-Methionine was kindly supplied by the Dow Chemical Company.

N-Acetyl-DL-methionine.—Acetylation with acetic anhydride and aqueous alkali as previously described[3] gave yields of 78–84% but the considerable solubility of the product required prolonged extraction with chloroform to secure maximum yields. Acetylation as by Knoop and Blanco[19] gave crude yields of about 80% but the discolored product was difficult to purify. The best method was a modification of that of Kolb and Toennies.[20] Acetylation was effected in three hours at room temperature in 10 parts of acetic acid containing acetic anhydride (1.6 moles). The sirupy product remaining after evaporation of acetic acid *in vacuo* was crystallized directly from 1.5 parts of water. Overnight 75–80% of the calculated amount of substantially pure product (m.p. 114–115°) separated. Additional crops increased the yield to 85%. The later crops were discolored and contained some free methionine but recrystallization from ethyl acetate or acetone gave satisfactory material. Pure N-acetyl-DL-methionine forms large prisms from water, m.p. 114–115°; solubilities in water, 9.12; acetone, 10.0; ethyl acetate, 2.29; chloroform, 1.33; neut. equiv., 191.3 (calcd., 191.1). du Vigneaud and Meyer[21] reported m.p. 114–115°.

Resolution of N-Acetyl-DL-methionine.—The acetamino acid and (−)-α-fenchylamine (0.5 mole) were dissolved in 200 cc. of warm water; the seeded solution slowly deposited the salt of N-acetyl-DL-methionine as small prisms. Successive crops having $[\alpha]^{25}D$ −7.5° to −6.8° (*c* 4, water) were systematically recrystallized from about 1 part of water and eventually gave 70.6 g. (82%). The pure salt has m.p. 172–173°; solubility in water, 15.6; $[\alpha]^{25}D$ −7.5° (*c* 4, water); −22.6° (*c* 4, methanol). It may be recrystallized from 2-propanol but resolution in this or other common solvents was not satisfactory. The more soluble salt forms sirupy solutions and was not purified.

N-Acetyl-D-methionine.—The pure salt was decomposed with alkali and the amine extracted repeatedly with benzene as previously described.[3] The solution was carefully neutralized, evaporated to small volume and treated with the calculated amount of concentrated hydrochloric acid. The rather soluble N-acetyl-D-methionine partially crystallized. Additional crops contained similarly soluble sodium chloride but were purified by extraction with hot ethyl acetate or chloroform and crystallization from these solvents. The yield of well-crystallized compound was 66% but additional less distinctive fractions (16%) gave pure D-methionine on subsequent hydrolysis. The pure compound forms triangular aggregates of plates from water or ethyl acetate, m.p. 104–105°; neut. equiv., 191.5; solubilities in water, 30.7; acetone, 29.6; ethyl acetate, 7.04; chloroform, 6.43; $[\alpha]^{25}D$ +20.3° (*c* 4, water); $[\alpha]^{30}D$ +18.2° (same solution). The effect of temperature on the rotation values is noteworthy. N-Acetyl-D-methionine has not been previously described but a specimen of the antipode prepared from natural methionine was reported[21] to have m.p. 111–111.5°; $[\alpha]^{25}D$ −16.1° (water). Since our values were checked repeatedly on several samples, it seems likely that the sample from natural sources was slightly impure.

D-Methionine.—The pure acetyl derivative (5 g.) was hydrolyzed with 3 *N* hydrobromic acid and the amino acid isolated (88%) as described in other instances.[3,4] After washing with methanol it had $[\alpha]^{25}D$ +8.2° (*c* 0.8, water); −23.5° (*c* 3, 1 *N* HCl). A sample recrystallized from 67% ethanol had $[\alpha]^{27}D$ −23.3° (*c* 3, 1 *N* HCl); $[\alpha]^{25}D$ −24.0° (*c* 3, 5.99 *N* HCl) in close agreement with reported values.[5,17]

In later resolutions the procedure was abbreviated by omitting isolation of N-acetyl-D-methionine. The solution remaining after careful extraction of the amine was acidified with 3 equivalents of 48% hydrobromic acid, boiled for three hours and evaporated to dryness under reduced pressure. The hydrobromide was taken up in methanol and D-methionine precipitated and washed repeatedly with hot methanol. The main crop (71%) had $[\alpha]^{25}D$ −23.0° (*c* 3, 1 *N* HCl).

L-Methionine.—The mother liquors of the resolution gave 48 g. of mixed acetylmethionines ($[\alpha]^{29}D$ −14.2°, hence *ca.* 81% of L-form). Since the L-form is more soluble than the accompanying DL-form it could not be purified by crystallization. Free methionine, presumably formed by slight hydrolysis during the prolonged earlier operations, was also detected. The crude material accordingly was combined with (+)-α-fenchylamine and fractionation was conducted as for the antipode. Rotation values for the less soluble salt, however, were slightly low and free methionine was detected in some fractions. The salt (35 g.) was decomposed and L-methionine obtained by the abbreviated procedure. The rotation $[\alpha]^{25}D$ +22.5° (*c* 3, 1 *N* HCl) indicates about 98% purity. The procedure is not satisfactory but L-methionine presumably could be prepared as readily as the D-form by initial resolution with (+)-α-fenchylamine.

Attempted Resolution with Other Bases.—N-Acetyl-DL-methionine formed needles with (−)-α-phenylethylamine in water but only slight resolution occurred in common solvents. The quinine salt crystallized readily from acetone with partial resolution; acetylmethionine from head crops had $[\alpha]^{25}D$ −4.1° (*c* 1.8 water); m.p. 103–112.5°. The cinchonine salt was not crystalline. The brucine salt formed a crystalline mass when a sirupy solution in acetone was kept several weeks. Stirring with cold acetone, decantation and recrystallization from acetone gave 70% of a fairly uniform salt as coarse granules, $[\alpha]^{25}D$ −7.6° to −8.2° (*c* 4, methanol). Acetyl-L-methionine from this salt after crystallization from chloroform had m.p. 102–104°; $[\alpha]^{25}D$ −19.6° (*c* 4, water) but the method is rather tedious.

Direct Resolution of DL-Methionine (a).—The amino acid (14.9 g., 0.1 mole) and ammonium (+)-α-bromocam-

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(21) V. du Vigneaud and C. E. Meyer, *ibid.*, **98**, 245 (1932).

phor-π-sulfonate[22] (32.8 g., 0.1 mole) were dissolved in 100 cc. of warm 1 *N* hydrochloric acid. The solution soon deposited the essentially pure L-methionine salt (21.1 g., 85.5%) as narrow transparent prisms with $[\alpha]^{25}D$ +61.7° (*c* 4, water), not substantially changed (+61.5°) after one or more crystallizations from 2.5 parts of water with small loss; solubility in water 5.4. The salt is a hydrate, stable at ordinary temperatures in air or over calcium chloride. Analytical data accord closely with a 7:4 hydration ratio.

Anal. Calcd. for $(C_{15}H_{26}O_6NS_2Br)_4 \cdot 7H_2O$: C, 36.53; H, 6.04; H_2O, 6.42. Found: C, 36.68; H, 6.04; H_2O, 6.47, 6.44, 6.44.

The anhydrous salt has $[\alpha]^{25}D$ +65.8°.

Additional crops (14.8 g.) from the original solution had $[\alpha]^{25}D$ +82° to +84° and consisted principally of ammonium (+)-α-bromocamphor-π-sulfonate dihydrate, $[\alpha]^{25}D$ +84.3° (*c* 4, water). This salt is apparently less soluble than the corresponding D-methionine salt, leaving D-methionine hydrochloride in the final liquors.

L-Methionine.—Pure L-methionine salt (12.4 g.) was dissolved in 2 parts of hot water and the amino acid was precipitated by addition of ammonia to *p*H 6, followed by 4 volumes of methanol. Recrystallization from 80% methanol removed traces of ammonium bromocamphorsulfonate and gave L-methionine (78%) having $[\alpha]^{25}D$ +23.4° (*c* 3, 1 *N* HCl). Evaporation of the methanol from filtrates gave a solution of ammonium bromocamphorsulfonate suitable for re-use.

D-Methionine (6.6 g.) having $[\alpha]^{25}D$ −17.5° (about 84% D-form) was obtained similarly from the foot liquors of the resolution.

(b).—DL-Methionine (119.3 g., 0.8 mole) and ammonium (+)-α-bromocamphor-π-sulfonate (131.2 g., 0.4 mole) were dissolved in 600 cc. of water containing 0.8 mole of hydrochloric acid and the warm solution was seeded. The initial crop of L-methionine salt (145 g.) and further crops obtained by successive concentrations to 200 cc. totaled 182 g. One crystallization series from 2.5 parts of water gave 163 g. (83%) of pure L-methionine salt (hydrate) as large prisms, $[\alpha]^{25}D$ +61.5°. Decomposition of this salt with ammonia gave 44.2 g. (74% based on the DL-form) of L-methionine, $[\alpha]^{25}D$ +23.4° (*c* 3, 1 *N* HCl). The combined mother liquors from the resolution and recrystallization gave 60.5 g. of D-methionine, $[\alpha]^{25}D$ −19.2°.

(c).—D-Methionine (0.05 mole) from (b) was purified by use of ammonium (−)-α-bromocamphor-π-sulfonate.[13] Except for the sign of rotation the constants of the salt and amino acid were substantially identical to those given for the antipodes.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF FORDHAM UNIVERSITY]

The Preparation of Pyridine Azo Compounds[1]

BY ROBERT W. FAESSINGER AND ELLIS V. BROWN

Two simple and closely related methods for the preparation of 2- and 4-pyridineazo compounds are described, based upon the reactions of (a) the sodium derivative of 2-aminopyridine with *p*-nitrosodimethylaniline, and (b) the disodium derivative of nitrobenzene or *p*-substituted nitrobenzenes with 2-aminopyridine. By these methods, a variety of new azo compounds have been prepared in which the azo linkage is attached to the 2- position of the pyridine nucleus. Application of the above methods to 3-aminopyridine, aniline and α-naphthylamine did not produce the desired azo compounds.

In investigating the synthesis of pyridine analogs of *p*-dimethylaminoazobenzene (Butter-yellow), which were to be tested for carcinogenic activity, it was observed that 3-aminopyridine easily diazotized and then readily coupled with dimethylaniline to form pyridine-3-azo-*p*-dimethylaniline.[2]

On the other hand, when either 2-aminopyridine or 4-aminopyridine was treated in a similar manner, coupling with dimethylaniline failed to occur to form the expected pyridine azo compound.

Tschitschibabin[3] investigated the preparation of pyridine-2-azoaryl compounds. He successfully prepared pyridine-2-azo-α-naphthol and pyridine-2-azoresorcinol by the reaction of sodium pyridine-2-diazotate with an alcoholic solution of α-naphthol or resorcinol while bubbling CO_2 through the mixture. However, Tschitschibabin reported that no azo product was obtained when dimethylaniline was used. We have since experimentally verified this claim and have found that coupling of 2- and 4-aminopyridine with dimethylaniline also failed when these amines were diazotized with nitrosylsulfuric acid, amyl nitrite or with nitric acid and potassium metabisulfite. In addition, it was observed that *p*-nitrosodimethylaniline in glacial acetic acid does not couple with the pyridylamines in question.

Koenigs, Kinne and Weiss[4] have prepared pyridine-4-azo-*m*-phenylenediamine, pyridine-4-azoresorcinol and the dinitrate of pyridine-4-azo-*p*-dimethylaniline by the reaction of 4-aminopyridine with nitrosylsulfuric acid in concd. nitric acid, followed by coupling with the arylamines. Our investigations of this method on 2-aminopyridine and 4-aminopyridine indicate that this process is of no value for the preparation of the pyridine-azo-*p*-dimethylanilines.

Koenigs, Fieigang, Lobmayer and Zscharn[5] have reported the preparation of pyridine-4-azobenzene by the oxidation of pyridine-4-hydrazobenzene with nitrous acid. M. Martynoff[6] has prepared azo compounds in the benzene series with some outstanding results. By heating a mixture of an arylamine and an arylnitro compound with powdered sodium hydroxide, aryl azo compounds were obtained. This process, when applied to the pyridine series using 2-aminopyridine and *p*-nitrodimethylaniline, yielded a viscous, black oil which showed no tendency to crystallize.

A series of pyridine azoaryl compounds has been prepared by two somewhat related processes. In the first method, the sodium derivative of 2-aminopyridine reacted with *p*-nitrosodimethylaniline in dry toluene under an atmosphere of nitrogen to yield the desired product with the azo linkage at the 2-position on the pyridine nucleus. The same results, with a slightly lower percentage yield,

(1) Paper presented at the 118th Meeting, American Chemical Society, Chicago, Ill., Sept. 7, 1950.

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