The acetamido compound (100 mg.) was dissolved in 1 ml. of 8 *N* ethanolic hydrogen chloride solution and allowed to remain at room temperature for sixteen hours. After evaporation of the alcohol, concentrated hydrochloric acid (0.25 ml.) was added to the residue and the solution evaporated on a steam-bath. The 2-amino-4-ethylthiazole hydrochloride was recrystallized from a mixture of ethanol and acetone; m. p. 185–187°.[10]

Summary

Several β-*l*-aminoketones ("Mannich bases") have been brominated and the resulting bromoaminoketones have been converted to 2-aminothiazoles. The structures of the bromination products of two β-aminoketones have been proved by independent syntheses of the 2-aminothiazoles derived from them. α-Chloro-β-piperidinopropiophenone has been synthesized from ω-chloroacetophenone by the Mannich reaction.

GLENOLDEN, PENNSYLVANIA RECEIVED JULY 1, 1946

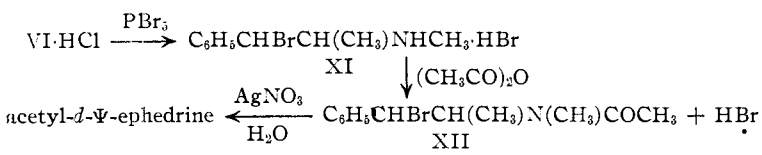
[CONTRIBUTION FROM THE U. S. FOOD AND DRUG ADMINISTRATION, FEDERAL SECURITY AGENCY]

The Constitution of Acetylephedrine and Acetyl-Ψ-ephedrine

BY LLEWELLYN H. WELSH

Acetyl-*l*-ephedrine was first prepared by Nagai[1] by the action of acetyl chloride on an ethereal solution of *l*-ephedrine (1-phenyl-2-methylamino-1-propanol), (I). He reported that the substance formed a hydrate, melted at 87°, and that he was unable to obtain crystalline salts from acid solutions of the compound. He represented it as an amide, or N-acetyl derivative of ephedrine.

Schmidt and Calliess[2] prepared acetyl-*d*-Ψ-ephedrine hydrochloride by refluxing acetic anhydride with *d*-Ψ-ephedrine hydrochloride, (VI·HCl), or its diastereoisomer, *l*-ephedrine hydrochloride (I·HCl). On treatment with alkali the product yielded acetyl-*d*-Ψ-ephedrine which, without proof, was represented as an N-acetyl derivative. Later,[3] Schmidt carried out a series of reactions which, according to his concepts, may be formulated in the following manner



Since XII, supposedly 1-bromo-1-phenyl-2-acetylmethylaminopropane, had no basic properties and yielded acetyl-*d*-Ψ-ephedrine by what was interpreted as replacement of bromine by hydroxyl when treated with aqueous silver nitrate, the N-acetyl structure (VII) was considered to be proved, and the corresponding hydrochloride was represented as an N-acetyl salt (VIII). Recently, however, Mitchell[4] has submitted evidence that the product formed by the action of acetic anhydride on XI is acetyl-*d*-Ψ-ephedrine hydrobromide instead of the compound represented by structure XII. This fact would render inconclusive Schmidt's evidence for an N-acetyl structure.

Mitchell[4] prepared acetyl-*l*-ephedrine and acetyl-*d*-Ψ-ephedrine by heating the corresponding alkaloids with one and one-half moles of acetic anhydride at 70° for ten minutes. From the Ψ-ephedrine derivative he prepared the well defined hydrochloride, but efforts to prepare acetyl-*l*-ephedrine hydrochloride yielded a poorly defined product contaminated with the diastereoisomer. On treatment with nitrous acid the acetylated alkaloids yielded nitrosoacetyl products of which the one derived from Ψ-ephedrine was well defined, whereas that resulting from acetyl-*l*-ephedrine apparently consisted of a mixture of diastereoisomers. Mitchell considered the ready formation of nitrosoacetyl compounds to be proof that the acetyl derivatives have a replaceable amino hydrogen and that they and the salts derived from them possess an O-acetyl structure. His interpretation of the reactions is expressed structurally in the following diagram.

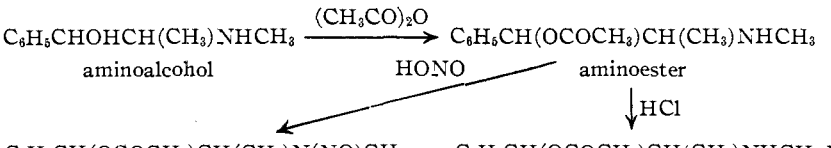
It seemed possible that criteria simpler than those used by Schmidt[3] and by Mitchell[4] could be employed to differentiate between N-acetyl (amide) and O-acetyl (ester) structures. If a compound under consideration is a hydroxyamide it should afford a practically neutral aqueous solution and should evince no titratable basicity under ordinary conditions. If it is an aminoester, however, it should yield an alkaline solution which, in the case of acetylephedrine and acetyl-Ψ-ephedrine, would be expected to be susceptible to quantitative titration with standard acid. It seemed possible also that the relationship between acetylephedrine and acetyl-Ψ-ephedrine on the one hand and the corresponding salts on the other might be that which exists between an N-acyl-β-aminoalcohol and the salt of the isomeric O-acyl-β-aminoalcohol. Although stable esters

(1) Nagai, *J. Pharm. Soc. Japan*, No. 127, 832 (1892). The author is indebted to Dr. J. G. Yoshioka for the translation of a portion of this publication which does not appear to be indexed in the Western literature, although reference to it and acetyl-*l*-ephedrine is made in a review by Chen and Kao, *J. Am. Pharm. Assoc.*, **15**, 625 (1926).

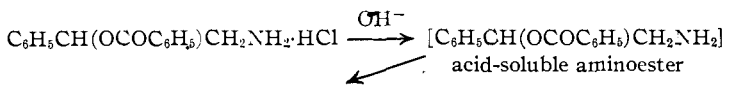
(2) Schmidt and Calliess, *Arch. Pharm.*, **250**, 154 (1912); see also Calliess, *Deut. Apoth. Z.*, **25**, 677 (1910).

(3) Schmidt, *ibid.*, **252**, 111 (1914).

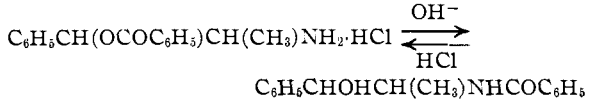
(4) Mitchell, *J. Chem. Soc.*, 1153 (1940).



may be formed from compounds in which an amino and a hydroxyl group are on adjacent carbon atoms,[5] it has been recognized for a number of years that aminoesters having an aryl group on the carbon atom bearing the acyloxy group rearrange with extreme ease to form hydroxyamides, and are stable only as salts. For example, when alkali is added to 1-phenyl-1-benzoyloxy-2-aminoethane hydrochloride an oil precipitates which, although initially partly soluble in acid, on crystallizing is found to consist of an acid-insoluble hydroxyamide[6]



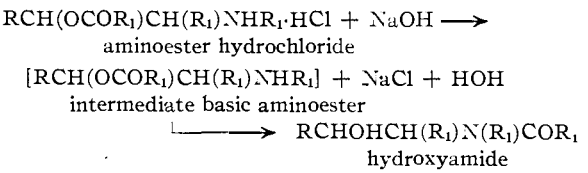
1-Phenyl-1-benzoyloxy-2-aminopropane hydrochloride when treated with alkali yields 1-phenyl-2-benzamido-1-propanol,[7,8] and on heating the hydroxyamide with concentrated hydrochloric acid the benzoyl group undergoes an N→O shift to reform an ester hydrochloride.[7]



The same N→O and O→N shifts of the benzoyl group have been observed in the N- and O-benzoyl derivatives of 1-(β-naphthyl)-2-aminoethanol, $C_{10}H_7CHOHCH_2NH_2$,[9] and reversible migration of acetyl also has been reported in a series of 1-aryl-2-(amino and hydroxylamino)-1-propanol derivatives.[10]

The investigation reported in the present paper was undertaken in the light of the foregoing considerations.

Acetylephedrine and acetyl-Ψ-ephedrine form aqueous solutions which are practically neutral, and by ordinary methods show no titratable basicity.[11] When the substances are dissolved in benzene and an ethereal solution of hydrogen chloride is added there immediately precipitate in about 98% yields relatively stable crystalline compounds having the composition of one mole of acetyl derivative and one mole of hydrogen chloride. These hydrogen chloride adducts differ sharply in properties from the salts of typical aliphatic amines in that their aqueous solutions have a strongly acid reaction and in that the combined hydrogen chloride may be directly and quantitatively titrated with alkali in the presence of methyl red or phenolphthalein. The unaltered original acetyl derivatives may be recovered quantitatively by extraction methods after adding excess alkali to aqueous solutions of the adducts. These facts make it obvious that acetylephedrine and acetyl-Ψ-ephedrine have an N-acetyl structure and not that of an aminoester. The adducts are regarded as N-acetyl hydrochlorides. On heating at 110° they are converted into isomeric substances. N-Acetyl-*d*-Ψ-ephedrine hydrochloride (VIII) thus gives a practically quantitative yield of a product (IX) of m. p. 179.5–181°, $[\alpha]^{20}D$ +98.6° (water), which is identical with the acetyl-*d*-Ψ-ephedrine hydrochloride prepared as described by Schmidt and Calliess.[2] Its aqueous solution shows a *p*H of 5 and cannot be directly titrated with alkali in the presence of methyl red as can those of the N-acetyl hydrochlorides. However, on addition of excess alkali and back-titrating it is found that one mole of alkali has been consumed with the simultaneous formation of one mole of N-acetyl-*d*-Ψ-ephedrine (VII). These facts can be reconciled only with an O-acetyl structure for IX and an O→N acetyl migration according to the equation



N-Acetyl-*l*-ephedrine hydrochloride (III) when heated yields a mixture of IX and O-acetyl-*l*-ephedrine hydrochloride (IV) by virtue of a Walden inversion occurring in the configuration about the (number one) carbon atom adjacent the benzene ring. This was demonstrated by the isolation of IX to the extent of 52% of the product from III and by the isolation of O-acetyl-*dl*-ephedrine hydrochloride (*dl* IV), m. p. 201–201.5° (dec.), to the extent of 23% of the product from N-acetyl-*dl*-ephedrine hydrochloride (*dl* III). The use of a racemic substance to demonstrate the presence of O-acetylephedrine hydrochloride was necessary because, so far, all efforts to isolate crystalline IV have been un-

(5) Cope and Hancock, THIS JOURNAL, **66**, 1448 (1944); Fourneau, *Bull. soc. chim.*, **11**, 141 (1944).

(6) Wolfheim, *Ber.*, **47**, 1440 (1914).

(7) Kanao, *J. Pharm. Soc. Japan*, **48**, 1070 (1928); German abstract, *ibid.*, p. 145.

(8) Hartung, Munch and Kester, THIS JOURNAL, **54**, 1526 (1932).

(9) Immediata and Day, *J. Org. Chem.*, **5**, 512 (1940)

(10) Bruckner and co-workers, *Ann.*, **518**, 226 (1935); *Arch. Pharm.*, **273**, 372 (1935); *J. prakt. Chem.*, **143**, 287 (1935); **148**, 117 (1937); **151**, 17 (1938); von Fodor, *Ber.*, **76B**, 1216 (1943).

(11) The formation of salts from these substances, however, was interpreted by Mitchell[4] as "direct neutralization."

successful. This compound appears to be much more soluble and difficultly crystallizable than the substances present in mixtures resulting from reactions which obviously lead to its formation. Polarimetric examination of the mixture of *l*-ephedrine and *d*-Ψ-ephedrine hydrochlorides obtained after alkaline hydrolysis of the rearrangement product from N-acetyl-*l*-ephedrine hydrochloride indicated that the inversion amounted to about 67%. O-Acetyl-*dl*-ephedrine hydrochloride, like the Ψ-ephedrine analog, yields an aqueous solution of *p*H 5 which cannot be titrated directly with alkali in the presence of methyl red, but which in the presence of excess alkali consumes one mole of base while forming one mole of N-acetyl-*dl*-ephedrine (*dl* II). It may be prepared in 50–73% yields by refluxing *dl*-ephedrine hydrochloride (*dl* I·HCl) with a mixture of acetyl chloride and acetic anhydride (5:1) or in about 75% yield by allowing a solution of *dl* II and hydrogen chloride in 90% acetone to stand at room temperature for six days during which an N→O shift of acetyl occurs. Under the latter conditions O-acetyl-*d*-Ψ-ephedrine hydrochloride is formed from VII. The formation of an N-acetyl hydrochloride as an intermediate preceding the rearrangement undoubtedly occurs in each case.

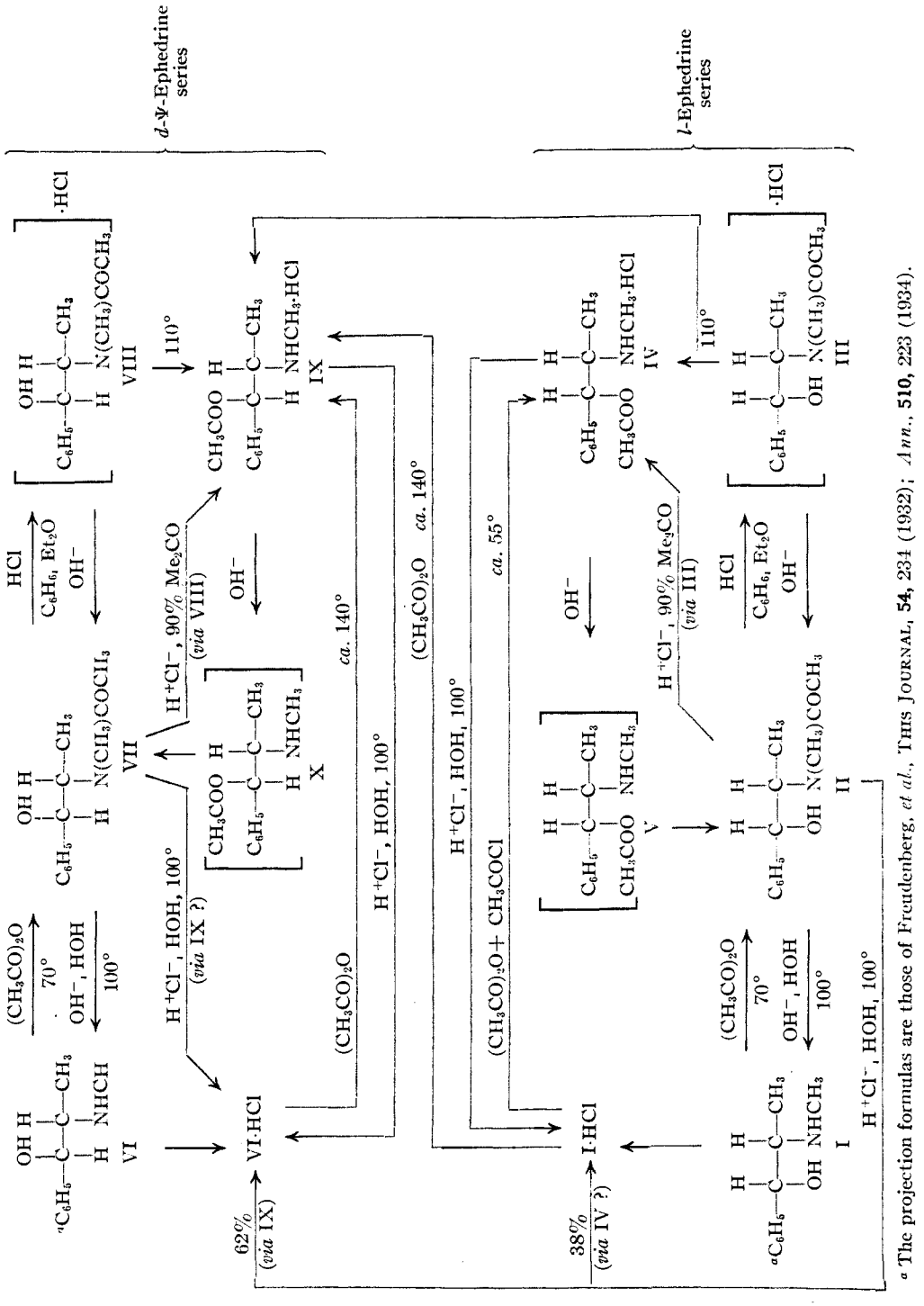
As stated previously, the O-acetyl salts cannot be titrated with alkali in the presence of methyl red. Under the experimental conditions addition of one drop of 0.1 *N* alkali to solutions of these substances yields solutions which are alkaline to this indicator (*p*H range *ca.* 4–6). In the case of O-acetyl-*d*-Ψ-ephedrine hydrochloride the yellow color gradually shifts toward the orange in the course of thirty minutes, whereas with the *dl*-ephedrine analog the yellow persists for a much longer period of time. In the presence of phenolphthalein (*p*H range *ca.* 8–10) a considerably larger volume of alkali can be added to a solution of an O-acetyl salt before an alkaline reaction, as evidenced by a slowly fading pink color, is produced. Addition of subsequent portions of alkali to a solution of O-acetyl-*d*-Ψ-ephedrine hydrochloride produces a pink color which persists for about twenty seconds, and a permanent end-point is reached only after one equivalent has been added. A solution of O-acetyl-*dl*-ephedrine hydrochloride gives with each subsequent increment of alkali a pink color which does not fade completely until about fifteen minutes have elapsed. A permanent end-point results when one equivalent of alkali has been added.

These observations lead to the conclusion that the intermediate basic aminoester of the Ψ-ephedrine configuration (X) rearranges much more rapidly to the hydroxyamide than does the one having the configuration of ephedrine (V), and that the speed of rearrangement in each case increases as the *p*H is raised. If, instead of adding alkali in portions until one equivalent is consumed, excess base is added at once, the rearrangement of both diastereoisomers occurs practically instantaneously.

Mitchell[4] effected the hydrolysis of N-acetyl-*l*-ephedrine (II) and its diastereoisomer by dilute acid and alkali. Only *d*-Ψ-ephedrine was isolable after either treatment of the Ψ-ephedrine derivative, the yield being about 97% in the presence of acid and 65% in an alkaline medium. Alkaline hydrolysis of N-acetyl-*l*-ephedrine gave only *l*-ephedrine in 76% yield, whereas acid hydrolysis provided an 88% yield of a mixture of hydrochlorides which was separated "by the ordinary methods" into approximately two parts of *l*-ephedrine and one part of *d*-Ψ-ephedrine hydrochloride. His work was repeated in this Laboratory but was carried out under quantitative conditions and was extended to include acid hydrolysis of the O-acetyl hydrochlorides. Quantitative yields of the 1-phenyl-2-methylamino-1-propanol having the configuration of the original molecule were obtained in all cases except in the acid hydrolysis of N-acetyl-*l*-ephedrine. In this case inversion occurred at the number one carbon atom, and the product consisted of a mixture of ephedrine and Ψ-ephedrine hydrochlorides. Its optical rotation indicated a composition of 61.7% of the latter and 38.3% of the former. This was confirmed substantially by the separation of the mixture into its components. Separation was achieved by taking advantage of the well-known difference in solubilities of the hydrochlorides in chloroform and of the free bases in water: thus it was possible to separate the diastereoisomers in quantities totalling 96.2–97.0% of the original mixture. Of this percentage *d*-Ψ-ephedrine fractions totalled 63–65% and those of *l*-ephedrine amounted to 35–37%. The ratio of the amounts of these substances is, therefore, almost the reverse of that reported by Mitchell.[4] It is of interest, however, that it corresponds approximately to the 60% inversion indicated in the results of Emde[12] who studied the effect of 25% hydrochloric acid on ephedrine at 100°. From a preparative point of view, the formation of Ψ-ephedrine by acetylation and subsequent hydrolysis with dilute acid is simpler and more efficient than the classical method which utilizes the action of strong hydrochloric acid on ephedrine. In the former method hydrolysis may be carried out directly on the acetylation mixture, and more than 60% inversion is effected in little more than an hour, whereas in the latter method about sixty hours of heating in a sealed tube is necessary to obtain approximately the same result.[12]

Since O-acetylephedrine hydrochloride undergoes deacetylation without inversion in the presence of dilute acid, inversion which occurs during acid hydrolysis of the N-acetyl derivative

(12) Emde, *Helv. Chim. Acta*, **12**, 377 (1929).



[a] The projection formulas are those of Freudenberg, *et al.*, THIS JOURNAL, **54**, 234 (1932); *Ann.*, **510**, 223 (1934).

must do so during hydrolytic fission of the N-acetyl group or during an N→O shift of acetyl prior to hydrolysis. Direct proof of the transitory existence of O-acetyl derivatives in the system would be difficult. However, indirect proof of the latter mechanism for the inversion may be considered as furnished by the facts that hydrolytic fission of the N-acetyl group would occur at a point relatively remote from the asymmetric atom involved, and that alkaline hydrolytic conditions, which could not involve an N→O shift, cause no inversion. Kanao[7] has observed a like inversion during the N→O migration of acyl radicals in derivatives of 1-phenyl-2-amino-1-propanol (norephedrine). Thus N-benzoylnor-*dl*-ephedrine yielded O-benzoylnor-*dl*-Ψ-ephedrine

hydrochloride, the only isolable product, on being heated with concentrated hydrochloric acid. The extent of inversion occurring during N→O acyl migrations in compounds of this type seems to depend on the conditions, notably temperature, which prevail during the acyl shift. Increase in temperature favors inversion as evidenced by the results of Kanao in the norephedrine series and by the predominance of inverted material in the product from the action of hot dilute acid on N-acetylephedrine. At lower temperatures less inversion takes place, since N-acetylephedrine yields as much as 74% of O-acetyl hydrochloride of the same configuration by the action of hydrochloric acid at room temperature in 90% acetone. These results roughly parallel those in which the hydroxyl group is esterified by acetyl which is provided by an extramolecular source: acetylation of *l*-ephedrine hydrochloride by boiling acetic anhydride[2] yields as much as 58% of O-acetyl-*d*-Ψ-ephedrine hydrochloride, whereas under the considerably milder conditions of refluxing with acetyl chloride–acetic anhydride (5:1) racemic ephedrine hydrochloride may furnish 73% of uninverted O-acetyl salt. No inversion occurs during the reverse (O→N) migration of acetyl in the ephedrine and Ψ-ephedrine derivatives even at 100°: addition of alkali to boiling aqueous solutions of O-acetyl-*dl*-ephedrine hydrochloride and the corresponding derivative of *d*-Ψ-ephedrine yielded only *dl*-ephedrine and *d*-Ψ-ephedrine, respectively. The occurrence of inversion during an O→N acyl migration in compounds of structures similar to those described in this paper apparently has not been recorded in the literature.

It is evident that the nitrosoacetyl derivatives prepared by Mitchell[4] owe their formation to an N→O migration of acetyl which accompanied the nitrosation. Any inversion which may have occurred during this reaction, and the formation of O-acetyl-*d*-Ψ-ephedrine hydrochloride in attempts to prepare a hydrochloride from acetyl-*l*-ephedrine are interpreted to be the result of such a migration, and could hardly result from a simple reaction involving only the amino nitrogen.

Further study of the acyl migrations is in progress.

Experimental[13]

Acetylation of Ephedrine and Ψ-Ephedrine.—Twenty grams of anhydrous *l*-ephedrine base was acetylated at 70° by Mitchell's procedure[4] with 20 cc. of acetic anhydride. The reaction mixture was dissolved in 250 cc. of water, 50 g. of sodium bicarbonate was added in small portions, with stirring, and the mixture allowed to stand, with occasional stirring, until the odor of the anhydride had disappeared. Enough water was then added to dissolve the salts present, and the N-acetyl-*l*-ephedrine was extracted with chloroform. After removal of chloroform, the residual oil slowly crystallized when triturated with cold 30–65° petroleum ether. The yield of crude was about 95%. It was dissolved in 40 cc. of warm benzene, and 40 cc. of 30–65° petroleum ether was added gradually, with stirring. Seeding and scratching caused slow deposition of the compound in the anhydrous form as the solution cooled to room temperature. The substance tends to crystallize out as aggregates of small prismatic forms, and too rapid cooling causes it to be precipitated initially as an oil. When no more material seemed to deposit, another 40-cc. portion of petroleum ether was added, the mixture was chilled for a couple of hours, filtered, and the precipitate was washed with petroleum ether. After drying several hours *in vacuo* over sulfuric acid it weighed 23.5 g. (93%), m. p. 85.5–86.5°; $[\alpha]^{20}D$ +8.1° (50% ethanol, c = 10),[14] +7.1° (abs. ethanol, c = 10), −63.2° (U. S. P. chloroform, c = 10). Mitchell[4] has reported m. p. 87° $[\alpha]^{20}D$ +7.0° (abs. ethanol).

N-Acetyl-*dl*-ephedrine was prepared in the same manner and yield as the *levo* compound, and recrystallized by adding 50 cc. of petroleum ether to its solution in 50 cc. of benzene as described above. The white powder appeared as aggregates of plates under the microscope: m. p. 77–78.5°.[15]

Anal. Calcd. for $C_{12}H_{17}NO_2$: N, 6.76. Found: N, 6.62.

Yields of crude N-acetyl-*d*-Ψ-ephedrine amounted to about 90% of the theoretical. About 87% recovery was obtained on forcing it out of benzene solution by adding petroleum ether (3 cc. of each solvent per gram of crude). The crystals consisted of small prismatic forms: m. p. 103.5–104°; $[\alpha]^{20}D$ +110.4° (50% ethanol, c = 2), +113.8° (abs. ethanol, c = 2), +121.8° (U. S. P. chloroform, c = 2). Values obtained by Mitchell[4] are m. p. 103–104°, $[\alpha]^{20}D$ +110.0° (abs. ethanol). Schmidt and Calliess[2] have reported a melting point of 101°.

N-Acetyl-*dl*-Ψ-ephedrine was recrystallized from benzene–petroleum ether in the manner described for the other N-acetyl compounds. It deposits slowly from supersaturated solutions as small prismatic forms, m. p. 76–77.5°.[15] This compound was prepared by Eberhardt[16] by adding alkali to O-acetyl-*dl*-Ψ-ephedrine hydrochloride, but he was unable to induce it to crystallize.

Anal. Calcd.: N, 6.76. Found: N, 6.58.

One per cent. aqueous solutions of each of the acetyl derivatives exhibited a *p*H of about 6.5, as did the distilled water used in preparing the solutions.

Preparation of the N-Acetyl Hydrochlorides.—Five grams of N-acetyl derivative was dissolved in 60 cc. of benzene, and 15 cc. of a 2.5 *N* solution of hydrogen chloride in absolute ether was added. The product from N-acetyl-*l*-ephedrine immediately precipitated in solid form, whereas those from the racemic substance and from N-acetyl-*d*-Ψ-ephedrine precipitated as thick oils which completely crystallized after a few minutes of trituration with a glass rod. After the mixture had stood a few minutes, any lumps were crushed, the mixture was filtered with suction, and the precipitate washed with benzene. The product was finely powdered under benzene, filtered and washed again. The filter cake was broken up and stirred frequently while the adherent benzene was allowed to evaporate during exposure to the air. The powder was left over sulfuric acid in a desiccator under diminished pressure (*ca.* 40 mm.) for two hours. Yields amounted to 96–100% of the theoretical.

N-Acetyl-*l*-ephedrine hydrochloride, so prepared, was

(13) Melting points are corrected.

(14) Tubes of 2-dm. length were used in all specific rotation determinations. The percentage of alcohol is expressed in per cent. by volume.

(15) Racemic acetylephedrine and acetyl-Ψ-ephedrine were first prepared in this laboratory by the addition of alkali to the O-acetyl hydrochlorides. The ephedrine derivative so obtained melted at 70–71°, whereas the Ψ-ephedrine derivative began to melt at 63°. After standing one year in stoppered containers the substances had melting points of 77–78.5° and 75–77.5°, respectively. All subsequent preparations of the compounds by this method and by acetylation of the appropriate base yielded products showing the higher melting point values. No explanation for the phenomenon is offered, except that it may be attributable to polymorphism.

(16) Eberhardt, *Arch. Pharm.*, **258**, 97 (1920).

a white powder which, under the microscope (430 ×), consisted of small prismatic forms. A capillary tube specimen when placed in a melting point bath and heated at a rate of 10° per minute to a temperature of 100°, and then at a rate of one-half degree per minute melted at 106–107°. A similar specimen placed in a bath at 100° and heated at a rate of 2 degrees per minute melted at about 110°. The $[\alpha]^{20}D$ was +5.6° (50% ethanol, c = 10). Aqueous solutions of the substance, as well as those of the other N-acetyl salts, possess a free acidity which can be directly titrated in the presence of phenolphthalein or methyl red.

Neut. equiv. Calcd. for $C_{12}H_{17}NO_2 \cdot HCl$: 243.7. Found: neut. equiv., 242.

A 0.5131-g. specimen was dissolved in water, a slight excess of 20% sodium hydroxide was added, and the solution was quantitatively extracted in a separatory funnel with five 10-cc. portions of chloroform according to the technique commonly used in the assaying of pharmaceutical preparations. The filtered extract was evaporated to dryness in a tared beaker which was then heated forty-five minutes at 105° and cooled in a desiccator. The residual resin of N-acetyl-*l*-ephedrine amounted to 0.4387 g. (1.006 molecular proportions). After being induced to crystallize, it melted at 85–86°.

N-Acetyl-*dl*-ephedrine hydrochloride appeared under the microscope as doubly refracting crystalline fragments. Capillary tube specimens, when placed in a bath at 45°, heated to 95° in four minutes, then heated at a rate of 1 degree per minute, formed a partial melt at about 100°. When further rapidly heated, fusion progressed as the temperature rose to 175°: slow heating from this point resulted in a clear melt at about 180°.

Neut. equiv. Calcd.: 243.7. Found: neut. equiv., 243.

N-Acetyl-*d*-Ψ-ephedrine hydrochloride was seen under the microscope as birefringent crystalline fragments. Specimens of the substance, contained in capillary tubes, when placed in a bath at 100° and heated at a rate of 5° per minute, underwent some sintering as the temperature rose to 175°. Heating at a rate of 1° per minute from this point resulted in the occurrence of fusion at *ca.* 180°. Specimens, plunged into a bath at 130°, in about fifteen seconds formed a partially fused mass which resolidified. Specimens placed in a bath at 120° underwent no more than sintering or softening. The substance showed $[\alpha]^{20}D$ +93.3° (c = 2, 50% C_2H_5OH). In absolute ethanol the rotation steadily decreased, probably as a result of rearrangement and deacetylation.

Neut. equiv. Calcd.: neut. equiv., 243.7. Found: 243.

By the same extraction procedure used on the N-acetyl-*l*-ephedrine salt, a 0.3028-g. specimen yielded 0.2581 g. (1.002 molecular proportions) of N-acetyl-*d*-Ψ-ephedrine of m. p. 103–103.5°.

O-Acetyl-*dl*-ephedrine Hydrochloride: A. From N-Acetyl-*dl*-ephedrine and Hydrochloric Acid–Acetone.—N-Acetyl-*dl*-ephedrine (2.00 g.) was dissolved in 20 cc. of acetone, 1.0 cc. of concentrated hydrochloric acid was added, and the container was stoppered and left at room temperature. Presence of seed crystals at this point did not cause precipitation, but addition of seed after eighteen hours caused immediate growth of crystals. After six days the precipitate was filtered off, washed with acetone and dried. The yield of crystals melting at 200°, with foaming, was 1.76 g. (74.8%) and was quite reproducible. Recrystallization from 95% ethanol gave a 93% recovery of material in the form of rectangular platelets, m. p. 201–201.5° (dec.). In melting point determinations the specimen was placed in a bath at 195° and heated at a rate of one degree per minute. The decomposition point was about two degrees lower if the bath was at room temperature at the start of the determination. A 2% aqueous solution had a *p*H of 5.0.

Anal. Calcd. for $C_{12}H_{17}NO_2 \cdot HCl$: Cl, 14.55. Found: Cl, 14.46.

The only crystalline material isolated in attempts to prepare the *l*-ephedrine derivative by this method was *l*-ephedrine hydrochloride. Occasionally a few crystals formed shortly after adding the hydrochloric acid, but these disappeared on standing, and undoubtedly were the N-acetyl hydrochloride.

A 0.1320-g. sample was dissolved in 10 cc. of water and one drop of methyl red indicator was added. Addition of one drop of 0.1 *N* sodium hydroxide caused the indicator to assume its yellow (alkaline) color which was unchanged for at least thirty minutes. Titration was attempted on a sample of the above size in the presence of 2 drops of phenolphthalein indicator. Alkali was added initially in 0.5-cc. portions. After addition of 1.5 cc. of 0.1 *N* base the solution assumed a faint pink color which persisted for a number of minutes. The alkali was subsequently added in approximately one-cc. portions. Each addition produced a pink to red color which in fifteen minutes faded completely. A red color which faded in ten to fifteen minutes to a permanent pink end-point was obtained after a total of 5.49 cc. (1.013 molecular proportions) of alkali had been added.

A 0.2406-g. sample of the compound was dissolved in 5 cc. of water, 20 cc. of 0.1 *N* sodium hydroxide was added, and the solution was backtitrated in the presence of methyl red. The elapsed time between the start of pipetting the base and the end of the titration was two minutes. The alkali consumed amounted to 9.82 cc. (0.995 molecular proportion).

A 0.5000-g. sample was dissolved in 5 cc. of water, 1 cc. of 20% sodium hydroxide was added, and the mixture was quantitatively extracted with six 10-cc. portions of chloroform. The residue from the filtered chloroform extracts was heated in an oven at 105° for one hour and cooled in a desiccator. The amorphous N-acetyl-*dl*-ephedrine amounted to 0.4223 g. (0.993 molecular proportion). After being induced to crystallize by seeding and rubbing it melted at 76–77.5°.

B. By Heating N-Acetyl-*dl*-ephedrine Hydrochloride.—N-Acetyl-*dl*-ephedrine hydrochloride (5.00 g.), contained in a small, loosely stoppered test-tube, was placed in an oven at 110° and left for seventy minutes. The resulting hard mass, which had a slight odor of hydrogen chloride and acetic acid, was powdered and 4.88 g. was dissolved in 20 cc. of boiling 95% ethanol. The solution was cooled to room temperature, and allowed to stand one hour. The crystals were filtered off, washed with a little cold ethanol, then with acetone and dried: 1.113 g. (22.8%), m. p. 200.5–201.5° (dec.).

C. From Acetyl Chloride–Acetic Anhydride and *dl*-Ephedrine Hydrochloride.—Five grams of powdered *dl*-ephedrine hydrochloride, 50 cc. of acetyl chloride and 10 cc. of acetic anhydride were refluxed together on the steam bath. As esterification proceeded the ephedrine salt became replaced by crystals of its acetyl derivative which tended to remain dispersed throughout the solution when boiling was interrupted, whereas unacetylated material collected together as a denser solid phase at the bottom of the flask. When no more ephedrine hydrochloride was observed (the time of reaction varied from five to six and one-half hours), 25 cc. of the liquid was distilled off on the steam-bath, the residual slurry was cooled and a mixture of 15 cc. of acetone and 30 cc. of ether was added. The suspension was cooled in ice, filtered, and the precipitate washed with acetone and dried. The yield varied from 3.0 to 4.4 g. (50–73%) of material melting at 201° (dec.). The lowest yields were associated with the longest periods of refluxing. Attempts to use acetyl chloride only as acetylating agent did not prove practical for the apparent reason that *dl*-ephedrine hydrochloride is not sufficiently soluble in the reagent.

Efforts to prepare O-acetyl-*l*-ephedrine by this method were unsuccessful.

O-Acetyl-*d*-Ψ-ephedrine Hydrochloride: A. From N-Acetyl-*d*-Ψ-ephedrine and Hydrochloric Acid-Acetone.—N-Acetyl-*d*-Ψ-ephedrine (0.25 g.) was dissolved in 2.5 cc. of acetone and 0.13 cc. of concentrated hydrochloric acid was added. In about one minute there began a deposition of crystals which grew to thick, irregular, hexagonal plates having a decided tendency to twin. Within two hours these had become markedly eroded. After five and one-

half hours, during which erosion progressed, the liquid was decanted off and seeded with O-acetyl-*d*-Ψ-ephedrine hydrochloride. Stout six-sided prisms, also having a tendency to twin, began to deposit. These were filtered off after a short while, washed with acetone and dried: m. p. 178.5–181°, not depressed when mixed with O-acetyl-*d*-Ψ-ephedrine hydrochloride prepared by other means, but greatly depressed when mixed with *d*-Ψ-ephedrine hydrochloride. In this experiment, the first precipitate was undoubtedly the N-acetyl hydrochloride which dissolved on contact with the mother liquor as the N-acetyl salt in solution rearranged to the O-acetyl salt. The method is of little preparative value since the latter compound deacetylates much more rapidly under the experimental conditions than does O-acetyl-*dl*-ephedrine hydrochloride. This was made apparent by an experiment in which double quantities of all the above materials were used. After deposition of the O-acetyl salt had been initiated and the system had been allowed to stand for eighteen hours, erosion of these crystals was noticed. After five days the quantity of solid had greatly diminished, and the appearance of a third crystalline substance was observed. After one month the mixture was filtered. The precipitate amounted to 0.33 g. (68%) of *d*-Ψ-ephedrine hydrochloride, m. p. and mixed m. p. 183–184°.

B. By Heating N-Acetyl-*d*-Ψ-ephedrine Hydrochloride.—N-Acetyl-*d*-Ψ-ephedrine hydrochloride (5.66 g.) was heated in the manner described for the *dl*-ephedrine analog. The solid mass was recrystallized from 60 cc. of propanol-2. The yield was 5.01 g. (88.4%),[17] of rectangular parallelepipeds, $n_\alpha = 1.525$, $n_\beta = 1.534$, $n_\gamma = 1.573$ (all ±0.003),[18] m. p. 179.5–181°, $[\alpha]^{20}D$ +98.6° (water, $c = 2$). Two additional recrystallizations failed to alter these constants significantly. A 2% aqueous solution showed a *p*H of 5.0. Schmidt and Calliess[2] reported m. p. 176°, $[\alpha]^{20}D$ +96.8° (water), whereas the values obtained by Mitchell[4] are m. p. 187°, $[\alpha]^{20}D$ +99.5° (water). The melting point of the compound varies somewhat with the rate of heating. Fairly reproducible results were obtained by placing capillary tube specimens in a bath preheated to 75°, raising the temperature to 175° in five minutes, and then heating at a rate of one degree per minute. Melting points reported in this paper for different batches of the substance were determined in this manner. Somewhat higher readings were obtained if the bath was preheated to 175°. Fusion of the specimen began at about 176° if the temperature of the bath was raised slowly from room temperature.

To explore the possibility that a few per cent. of O-acetyl-*l*-ephedrine hydrochloride might be formed during the rearrangement as a result of inversion, a 0.2012-g. specimen of VIII was heated as previously described. The loss of weight as a result of heating was less than 1 mg. The aqueous solution of the product consumed only 0.05 cc. of 0.1 *N* alkali on direct titration in the presence of methyl red, which shows that no more than a very small amount of N-acetyl salt was present after the heating. Alkaline hydrolysis by the method described in a subsequent section gave a quantitative yield (0.168 g.) of *d*-Ψ-ephedrine hydrochloride, m. p. 183–184°, $[\alpha]^{21}D$ +61.0° (water, $c = 1.5$). The melting point and rotation indicate that if any inversion occurred, its extent was quite small.

A 0.1354-g. sample was dissolved in 10 cc. of water and one drop of methyl red indicator was added. Addition of 1 drop of 0.1 *N* sodium hydroxide produced a yellow color in the solution. In thirty minutes the color had definitely shifted toward the neutral, orange color of the indicator. A duplicate weight of substance was directly titrated in the presence of 2 drops of phenolphthalein indicator. The 0.1 *N* alkali was added in 1-cc. portions, and each of four such additions produced a transient pink color which disappeared almost as soon as the flask was swirled. After addition of the fifth portion a period of about twenty seconds elapsed before disappearance of color. Alkali was then added in two-drop, and, finally, one-drop portions, with intervals of twenty seconds between additions. A permanent pink end-point was obtained when 5.59 cc. (1.005 molecular proportions) of alkali had been added. The compound behaved in the same manner as did O-acetyl-*dl*-ephedrine hydrochloride on addition of excess alkali and back-titrating in the presence of methyl red.

A solution of 0.4512 g. of the substance in 5 cc. of water was made alkaline and quantitatively extracted with chloroform. The residue of N-acetyl-*d*-Ψ-ephedrine obtained amounted to 0.3847 g. (1.00 molecular proportion), m. p. 102.5–103.5°, $[\alpha]^{20}D$ +120.5° (U. S. P. chloroform, $c = 3.5$).

C. By Heating N-Acetyl-*l*-ephedrine Hydrochloride.—N-Acetyl-*l*-ephedrine hydrochloride (5.00 g.) was placed in an oven at 110°. After about forty minutes it had completely liquefied, and after an additional thirty minutes the melt had partly resolidified. It was digested with 50 cc. of hot benzene, cooled to room temperature, and allowed to stand fifteen minutes. The insoluble crystals were filtered off, washed with benzene and dried. There resulted 2.58 g. (51.6%) of O-acetyl-*d*-Ψ-ephedrine hydrochloride, m. p. 175.5–177.5°. Recrystallization from propanol-2 yielded 2.26 g. of product melting at 179–180.5°. No well-defined crystalline material was obtained from the filtrate from the benzene digestion.

To ascertain polarimetrically the extent of inversion which occurs during the rearrangement, a 0.5062-g. sample contained in an open 50-cc. round-bottom flask was placed in an oven at 110°. The sample was completely liquefied after twenty-five minutes, and was kept at 110° an additional eighty minutes. The product was subjected to the alkaline hydrolysis procedure described in a subsequent section. The yield of mixed ephedrine and Ψ-ephedrine hydrochlorides obtained from the ether extract was 0.4175 g. (99.7%), $[\alpha]^{20}D$ +29.7° (water, $c = 3.8$). The rotation corresponds to 66.7% *d*-Ψ-ephedrine hydrochloride. In calculating the composition of the mixture, values of +61.7° and −34.5° were used for the *d*-Ψ- and *l*-ephedrine hydrochlorides, respectively, and represent results obtained in this laboratory from recrystallized materials.

D. From Acetic Anhydride and *l*-Ephedrine Hydrochloride.[2]—*l*-Ephedrine hydrochloride (5.00 g.) was refluxed three hours with 50 cc. of acetic anhydride. The mixture was evaporated to a sirup on the steam-bath in a current of air, and 30 cc. of acetone was added. Since deposition of crystals was slow, the mixture was left two days in a refrigerator freezing unit. The crystals were washed with acetone, then ether, and dried. The crop of product melting at 179.5–181° was 2.32 g. A second crop of 1.18 g., m. p. 177.5–179.5°, was obtained by adding more ether to the filtrate and washings. The total yield amounted to 57.9%. Recrystallization from propanol-2 yielded 3.17 g. (52.5%) of substance melting at 178.5–180.5°. It gave no depression of melting point when mixed with the products obtained in **A, B** and **C**. When the reaction was carried out on *dl*-ephedrine hydrochloride the yield of racemic product,[16] melting at 168–170° was about 40%. Neither Schmidt and Calliess[2] nor Eberhardt[16] reported yields.

Acid Hydrolysis: A. N-Acetyl-*l*-ephedrine.—A mixture of 5.00 g. of N-acetyl-*l*-ephedrine and 100 cc. of 5% hydrochloric acid was refluxed one hour. The solution was distilled to apparent dryness under a water-pump vacuum (bath temperature 50–60°, raised to 100° toward end of distillation) and the residue was dried at 105°. Removal of water and acid at a relatively low temperature was carried out in order to minimize any inversive or decomposing effect which might be manifest under more vigorous conditions as the acid concentration increased during distillation. The yield of mixed hydrochlorides was quantitative (4.86 g.). The mixture began to melt at 160°, and showed $[\alpha]^{20}D$ +24.9° (water, $c = 2$). This rotation corresponds to a composition of 61.7% of *d*-Ψ-ephedrine hydrochloride and 38.3% of *l*-ephedrine hydrochloride. A mixture prepared to contain these percentages of pure hydrochlorides

(17) The percentage recovery on recrystallization is in the same range as those obtained from purified material and indicates the reaction is practically quantitative.

(18) Refractive indices by Wm. V. Eisenberg, Division of Microbiology, U. S. Food and Drug Administration.

of ephedrine and Ψ-ephedrine began to melt at 161°, and showed $[\alpha]^{20}D$ +25.0° under the same conditions. The dried reaction product was refluxed with 120 cc. of U. S. P. chloroform for one-half hour, and the suspension was cooled to room temperature and allowed to stand one-half hour. The crystals of *l*-ephedrine hydrochloride were filtered off, washed with chloroform and dried: 1.128 g. (23.2%), m. p. 214–216°. The residue obtained from the filtrate and washings was dissolved in 10 cc. of water, 5 cc. of 20% sodium hydroxide was added, and the mixture was kept at 0° for one-half hour. The precipitate of *d*-Ψ-ephedrine base was filtered off, washed with 20 cc. of ice-water and dried overnight over sulfuric acid: 2.366 g. (59.4%), m. p. 117.5–118.5°. The filtrate and washings were quantitatively transferred to a separatory funnel, saturated with anhydrous sodium sulfate, and quantitatively extracted with five 20-cc. portions of chloroform. A slight excess of ethereal hydrogen chloride was added to the filtered extract, and the suspension was evaporated to dryness. The residue of hydrochlorides was treated as before with 40 cc. of chloroform, the suspension was filtered, and the ephedrine hydrochloride was washed with chloroform: 0.522 g. (10.7%), m. p. 214.5–216°. The residue from the chloroform filtrate was dissolved in 1.5 cc. of water and filtered to remove a small amount of insoluble matter. The filtrate plus washings (volume 3.5 cc.) was made alkaline with 0.5 cc. of 20% sodium hydroxide, and the crop of Ψ-ephedrine was filtered off, washed with cold water and dried: 0.096 g. (2.4%), m. p. 117–118°. A third crop of *l*-ephedrine hydrochloride was obtained by benzene extraction of the filtrate (saturated with sodium sulfate), addition of ethereal hydrogen chloride to the extract, and digestion of the mixed hydrochlorides with chloroform: 0.050 g. (1.0%), m. p. 207.5–213.5°. The combined Ψ-ephedrine fractions showed $[\alpha]^{20}D$ +51.6° (abs. ethanol, c = 5), and a neutralization equivalent of 166.6 (calcd. 165.2). A composite of the three *l*-ephedrine hydrochloride fractions gave $[\alpha]^{20}D$ −32.2° (water, c = 10). Emde[19] reported $[\alpha]^{17}D$ +53° for *d*-Ψ-ephedrine in absolute ethanol.

Hydrolysis was also carried out directly on reaction mixtures resulting from the acetylation of ephedrine base. There was no appreciable difference between the results obtained from such mixtures and the results from the hydrolysis of purified material. In either case the total recoveries were 96.2–97.0% of product consisting of 62.6–65.3% of *d*-Ψ-ephedrine and 34.7–37.4% *l*-ephedrine. Hydrolysis with 25% hydrochloric acid gave somewhat lower yields and was accompanied by the formation of material having a terpenoid odor.

B. O-Acetyl-*dl*-ephedrine Hydrochloride.—A solution of 1.176 g. of the substance in 20 cc. of 5% hydrochloric acid was refluxed one hour and the liquid distilled off under a water-pump vacuum. The residue was dried at 105°. The yield of *dl*-ephedrine hydrochloride was quantitative (0.978 g.), m. p. 187.5–189.5°. Digestion with 10 cc. of chloroform resulted in a 1% loss of weight and raised the melting point to 188.5–190° (no depression on mixed m. p.).

C. N-Acetyl-*d*-Ψ-ephedrine.—The hydrolysis mixture from 0.5001 g. of substance and 10 cc. of acid was quantitatively transferred to a separatory funnel, the solution (volume now 30 cc.) was saturated with sodium sulfate and made alkaline with 8 cc. of 20% sodium hydroxide. The mixture was extracted quantitatively with six 25-cc. portions of ether, and the combined extracts were washed with three 4-cc. portions of water. The water washings, after being combined, were shaken with 15 cc. of ether which was then added to the main ether extract, and the whole was filtered through cotton. A slight excess of ethereal hydrogen chloride was added and the mixture was evaporated to dryness on the steam-bath in a current of air. After drying at 105° the residue of *d*-Ψ-ephedrine hydrochloride weighed 0.4854 g. (99.7%), m. p. and mixed m. p. 183–184°, $[\alpha]^{20}D$ +61.2° (water, c = 3).

D. O-Acetyl-*d*-Ψ-ephedrine Hydrochloride.—The hydrolysis product from 0.5510 g. of material was subjected to the procedure described in **C**. A quantitative yield (0.4562 g.) of *d*-Ψ-ephedrine hydrochloride was obtained, m. p. and mixed m. p. 183–184°, $[\alpha]^{20}D$ +61.5° (water, c = 3).

Alkaline Hydrolysis: A. N-Acetyl-*l*-ephedrine.—A 0.5000-g. sample was dissolved in 1 cc. of 95% ethanol, 10 cc. of 10% sodium hydroxide was added, and the mixture was refluxed four hours. The hydrolyzate was transferred to a separatory funnel by means of benzene and 10 cc. of water, and extracted quantitatively with six 20-cc. portions of benzene. The *l*-ephedrine hydrochloride obtained from the extracts weighed 0.4850 g. (99.7%), m. p. 217.5–218.5°, $[\alpha]^{20}D$ −34.8° (water, c = 4).

B. N-Acetyl-*d*-Ψ-ephedrine.—Hydrolysis of 0.5000 g. as in **A**, and extraction of the Ψ-ephedrine into ether instead of benzene, ultimately gave a quantitative yield (0.4880 g.) of *d*-Ψ-ephedrine hydrochloride, m. p. 183–184°, $[\alpha]^{20}D$ +61.9° (water, c = 3).

Rearrangement of O-Acetyl Hydrochlorides at 100°.—Samples (0.5000 g.) of O-acetyl-*dl*-ephedrine hydrochloride and the *d*-Ψ-ephedrine analog were dissolved in a mixture of 5 cc. of water and 1 cc. of 95% ethanol, and the solution was heated to boiling, under reflux. Five cc. of 20% sodium hydroxide was added dropwise through the condenser, and refluxing was continued for four hours in order to ensure complete deacetylation. The hydrolyzates were worked up respectively as described in **A** and **B** of the preceding section. Thus was obtained 0.4115 g. (99.4%) of *dl*-ephedrine hydrochloride, m. p. 188.5–189.5°, and 0.4118 g. (99.5%) of *d*-Ψ-ephedrine hydrochloride, m. p. 183–184°.

(19) Emde, *Helv. Chim. Acta*, **12**, 365 (1929).

Summary

1. Acetylephedrine and acetyl-Ψ-ephedrine have been shown to possess an N-acetyl (amide) structure. The acetyl-*d*- and -*dl*-Ψ-ephedrine hydrochlorides previously reported in the literature are O-acetyl (ester) hydrochlorides.

2. Acetylephedrine and acetyl-Ψ-ephedrine form, under suitable conditions, relatively stable hydrogen chloride adducts (N-acetyl hydrochlorides) which, when heated, undergo an N→O shift of acetyl and rearrange to O-acetyl hydrochlorides. The rearrangement of the Ψ-ephedrine derivative occurs without appreciable inversion at the carbon atom α to the phenyl group, whereas the product of the rearrangement of the ephedrine analog is a mixture of diastereoisomers in which the Ψ-ephedrine derivative predominates.

3. Treatment of N-acetyl-Ψ-ephedrine and the O-acetyl hydrochlorides of ephedrine and Ψ-ephedrine with boiling 5% hydrochloric acid yields only the aminoalcohol having the configuration of the original compound. N-Acetylephedrine yields a mixture of ephedrine and Ψ-ephedrine hydrochlorides in the approximate ratio of 38:62. The inversion which occurs does so during an N→O shift of acetyl prior to hydrolysis, and does not result from the action of the acid on ephedrine.

Alkaline hydrolysis causes no inversion in any case.

4. The extent of the inversion which accompanies an N→O shift of acetyl in the ephedrine series appears to increase with increasing temperature.

5. In the presence of alkali the O-acetyl hydrochlorides rearrange quantitatively to N-acetyl compounds without change in configuration.

The rearrangement undoubtedly takes place through an intermediate O-acetyl base, and is much more rapid in the Ψ-ephedrine derivative than in the one having the configuration of ephedrine. The speed of rearrangement is affected by the hydrogen ion concentration, and is practically instantaneous at a sufficiently high *p*H.

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Neovitamin A[1]

BY CHARLES D. ROBESON AND JAMES G. BAXTER

It was previously reported that a portion of the vitamin A in the unsaponifiable matter of fish liver oils could not be crystallized.[2] This so-called "noncrystallizable vitamin A" has been further studied and from it has been isolated in crystalline form a previously unrecognized geometric isomer of vitamin A, which we shall call "neovitamin A." Our work indicates that neovitamin A differs from vitamin A only in the spacial configuration about the double bond nearest the hydroxyl group.

The new isomer constitutes about 35% of the total vitamin present in a number of the common fish liver oils, so that its physical and chemical properties, and especially its biological potency, are of commercial as well as theoretical interest. This paper is concerned with these properties, with the method of isolation, and with the structure of the newly recognized vitamin. A preliminary report on the work has already been published.[3]

Isolation of Crystalline Neovitamin A.—Some of the preliminary steps in the isolation work have previously been described.[4] The source material was shark liver oil. This was distilled in a cyclic molecular still to concentrate the neovitamin A and vitamin A esters. The nonsaponifiable matter was prepared from the concentrate and redistilled to further concentrate the two vitamins. The major amount of the vitamin A was separated from this distillate by crystallization from ethyl formate at −70°. After removal of solvent from the filtrate an orange oil was obtained, from which substantially all the remaining vitamin A was removed by selective adsorption. Finally, the neovitamin A concentrate was esterified with phenylazobenzoyl chloride and the ester crystallized. This ester (m. p. 94–96°, Fig. 1) melted higher than the corresponding vitamin A ester (m. p. 79–80°, Fig. 2).

Saponification of the ester yielded neovitamin A as an oil which crystallized from a solution in ethyl formate at −35° as pale yellow needles (m. p. 58–60°). The crystals (Fig. 3) were markedly different in appearance than the yellow prisms of vitamin A (m. p. 62–64°, Fig. 4) and a mixed melting point determination showed a depression.

The crystalline anthraquinone β-carboxylate esters of neovitamin A and vitamin A were prepared and found to differ. The neo ester was red (m. p. 134–136°) while the vitamin A derivative was yellow (m. p. 121–122°). Hamano[5] and Mead[6] obtained red crystals (m. p. 118°) and yellow crystals (m. p. 123–124°) which they considered to be polymorphic forms of the same compound. It now seems probable that their red crystals, which melted 16–18° lower than ours, were a mixture of the neovitamin A and vitamin A esters, while their yellow crystals were pure vitamin A anthraquinonecarboxylate.

Physical and Chemical Properties of Neovitamin A

Ultraviolet Absorption Spectrum.—Neovitamin A has an absorption curve similar in shape to that of vitamin A but the position of the maximum (328 mμ) is slightly different from that of vitamin A (324–5 mμ, Fig. 5). An average value of $E^{1\%}_{1\,cm.}$ (328 mμ) = 1645 for neovitamin A was found (5 preparations).[7]

Infrared Spectrum.—The infrared transmission spectrum of neovitamin A is compared with that of vitamin A in Fig. 6. The curves are almost identical, with slight differences occurring in the position of the bands at 9.25 and 8.00 microns.[8]

Atmospheric Oxidation.—Neovitamin A was slightly more resistant to atmospheric oxidation than vitamin A when dissolved in refined cottonseed oil at a concentration of 20,000 U. S. P. units per gram. The palmitic esters of the two compounds were equally stable in refined cottonseed oil at a concentration of 90,000 units per gram. These conclusions were based on data obtained by uniformly exposing the oil solutions to air at 55° in an oven. A rocking device and glass rocker tubes provided, respectively, the agitation

(1) Presented in part at the Atlantic City Meeting of the American Chemical Society, Atlantic City, New Jersey, April 11, 1946.

(2) J. G. Baxter, P. L. Harris, K. C. D. Hickman and C. D. Robeson, *J. Biol. Chem.*, **141**, 991 (1941).

(3) C. D. Robeson and J. G. Baxter, *Nature*, **155**, 300 (1945).

(4) J. G. Baxter and C. D. Robeson, THIS JOURNAL, **64**, 2411 (1942).

(5) S. Hamano, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **32**, 44 (1937).

(6) T. H. Mead, *Biochem. J.*, **33**, 589 (1939).

(7) Mr. G. Wait and assistants of this Laboratory made the measurements using a Beckman spectrophotometer.

(8) The infrared transmission spectra were obtained by Dr. S. F. Kapff of this Laboratory, using a Perkin–Elmer infrared spectrometer, Model 12-A.