TABLE IV

INFRARED SPECTRA OF SOME BENZOYLCYCLOPENTANONES

Compound	X	Tautomer[a]	M.p., °C.	C=O unconj.	C=O conj.	C=C region
I	H	Keto[b]	Liq.	1742 s	1688 s	1640-1510[d]
VII	p-OCH_3	Enol[c]	70–72	1738 vw		1640 m, 1605 s, 1600 m
VII	p-OCH_3	Keto[b]	63–64	1740 s	1682 s	1605 s
VIII	p-NO_2	Enol[b]	79–80			1640 s, 1605 m
VIII	p-NO_2	Keto[b]	88–89		1685	1650, 1615

[a] No OH band in the 3000–4000-cm.$^{-1}$ region for either tautomer. [b] Colorless. [c] Yellow. [d] Hyphen indicates overlapping bands.

the resulting ether extract. Compounds II and XV were distilled; in all other cases employing this procedure the product crystallized directly from hexane, pentane, or ligroin

Isolation of 2,5-Dibenzoylcyclopentanone (XX).—When the preparation of I was carried out as above, the ether extract from the decomposed reaction mixture was flash distilled and the residue taken up in hot methanol. Cooling of the methanol yielded a trace of yellow crystals of XX,[1] m.p. 121–122°.

Alternative Benzoylation Methods. Acid Chloride.—A solution of 13.9 g. (0.165 mole) of cyclopentanone and 23.0 g. (0.165 mole) of benzoyl chloride in 200 ml. of dry benzene was stirred at 0° under dry nitrogen. Sodium hydride[20] (15 g., 0.33 mole) was added in ten portions with cooling and stirring. The mixture was refluxed for 3 hr. Decomposition and isolation in the usual manner yielded 4 g. of cyclopentanone (2,4-DNP, m.p. 143°), 6 g. of benzoyl chloride, and a few drops of I (b.p. 150–155°/7 mm.).

Methyl Ester Method.—A dispersion of 17.8 g. (0.33 mole) of sodium methoxide in 300 ml. of benzene was stirred as 13.9 g. (0.165 mole) of cyclopentanone was added over a 10-min. period. After 5 min. more, 22.5 g. (0.165 mole) of methyl benzoate was added over a 10 min. period. The mixture was allowed to stand for 0.5 hr. and heated on a steam bath overnight. The mixture was decomposed and the product isolated by procedure C above. Yellow crystals from *n*-hexane were identified as 2-benzoyl-5-cyclopentylidenecyclopentanone (XXI), 2 g., m.p. 99–100°.[10] No other product was isolated.

Phenyl Ester Method.—The procedure for preparation of aroylcyclohexanones[3] was applied to phenyl anisoate and cyclopentanone. Isolation procedure C above yielded 7 g. of copper chelate, from which 4 g. of 2-(*p*-anisoyl)-5-cyclopentylidenecyclopentanone (XXII) was isolated, m.p. 104–106°.

Anal. Calcd. for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 75.90; H, 7.52.

Determination of Percentage Enol.—The enol content was determined at equilibrium. A weighed sample of diketone (approximately 0.2 g.) was dissolved in 50 ml. of anhydrous absolute methanol. The solution was allowed to stand for 48 hr. at room temperature in the dark. The modified bromine titration[2,21] procedure was used to determine percentage enol. Due to a shifting end point, the titration was carried out rapidly, arriving at the end point within 2 min. for consistent results. The results of the determinations are listed in Table II.

Measurement of Spectra.—The infrared spectra were measured using a Perkin–Elmer Model 21 double-beam recording spectrophotometer with a sodium chloride prism. The control settings were maintained constant at: resolution, 926; response 1; gain, 5; speed, 4; suppression, 4. The concentration used was 40 mg./ml. Matched 0.1-mm. cells were used in standard double-beam operation. Data obtained are listed in Table IV.

(20) A 52% mineral oil dispersion was first washed with benzene in a dry nitrogen atmosphere to give oil-free sodium hydride, which was used immediately.

(21) W. T. Smith, Jr., and R. L. Shriner, "The Examination of New Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 101.

The Synthesis of Hydroxycotinine and Studies on Its Structure[1]

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Hydroxycotinine, an optically active metabolite which arises *in vivo* from (−)-nicotine by way of the intermediate (−)-cotinine, was converted to chlorocotinine by reaction with thionyl chloride. The resultant chlorocotinine yielded (−)-cotinine upon hydrogenolysis. γ-(3-Pyridyl)-γ-oxo-α-acetamidobutyric acid, prepared from acetamidomalonic ester and bromomethyl 3-pyridyl ketone, was converted with methylamine and hydrogen in the presence of Raney nickel to acetamidocotinine. Aminocotinine from hydrolysis of the latter afforded two isomeric pairs of hydroxycotinine. The dextrorotatory form obtained by resolution of one of these pairs corresponded in melting point, mixed melting point, and infrared absorption spectra to metabolic hydroxycotinine.

In the metabolism of (−)-nicotine in the dog, oxidation of the pyrrolidine ring leads to the formation of (−)-cotinine, 5-(3′-pyridyl)-1-methylpyrrolidone-2.[4] Metabolism of (−)-cotinine gives rise[5–7] in turn to a number of additional pyridino compounds which, through the ubiquitousness[8] of cotinine, may be common to a number of species.

During early studies[5] on the metabolism of (−)-cotinine in the dog a fraction giving a strong Koenig reaction and containing two components was obtained. One component was identified in crystalline form as (−)-demethylcotinine. The other metabolic component was acetylated with acetic anhydride in pyridine and then yielded a crystalline picrate, $C_{18}H_{17}N_5O_{10}$. The acetylated metabolite, acetoxycotinine, gave[7] upon acidic hydrolysis a colorless alcohol, hydroxy-

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(4) H. McKennis, Jr., L. B. Turnbull, and E. R. Bowman, *J. Am. Chem. Soc.*, **80**, 6597 (1958).

(5) H. McKennis, Jr., L. B. Turnbull, E. R. Bowman, and E. Wada, *ibid.*, **81**, 3951 (1959).

(6) H. McKennis, Jr., E. R. Bowman, and L. B. Turnbull, *Proc. Soc. Exptl. Biol. Med.*, **107**, 145 (1961).

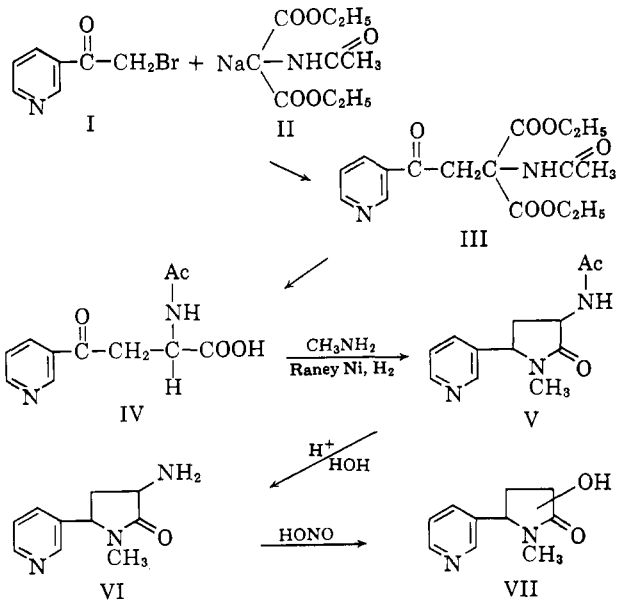
(7) E. R. Bowman and H. McKennis, Jr., *J. Pharmacol. Exptl. Therap.*, **135**, 306 (1962).

(8) (a) F. E. Guthrie, R. L. Ringer, and T. G.Bowery, *J. Econ. Entomol.*, **50**, 822 (1957); (b) H. B. Hucker, J. R. Gillette, and B. B. Brodie, *Nature*, **183**, 47 (1959); (c) R. Truhaut and M. de Clercq, *Bull. soc. chim. biol.*, **41**, 1693 (1959).

cotinine, $C_{10}H_{12}N_2O_2$, $[\alpha]^{20}D$ +42.5° (methanol). The alcohol in turn formed[7] a crystalline picrate, $C_{16}H_{15}N_5O_9$. Hydroxycotinine has also been obtained[9] from the urine of rats following administration of (−)-cotinine and has now been isolated, as the derivative acetoxycotinine picrate, from the urine of smokers. The strong Koenig reaction of hydroxycotinine and the failure of the compound to be retained on Dowex 21K (OH^-) led[5] to the provisional structure in which the metabolite bears an hydroxyl group on the pyrrolidone ring of the parent compound, cotinine. The present report, in which the total synthesis of hydroxycotinine is described, serves to bear out the correctness of this assignment.

Both 3- and 4-hydroxycotinine, 5-(3′-pyridyl)-3-hydroxy-1-methylpyrrolidone-2 and 5-(3′-pyridyl)-4-hydroxy-1-methylpyrrolidone-2 are attractive to consider as possible metabolites and possible inhibitors of the metabolism of nicotine and related substances. The apparent reversibility of the reaction cotinine →γ-(3-pyridyl)-γ-methylaminobutyric acid *in vivo*[4,10,11] can, furthermore, be hypothetically extended[12] to the hydroxy acids corresponding to 3- and 4-hydroxycotinine.

The synthesis of 3(?)-hydroxycotinine has now been completed by the following series of reactions:



Among several possible syntheses the foregoing was attractive since two of the important intermediates, 3-acetylpyridine, the intermediate for preparation of the bromomethyl pyridyl ketone, and acetamidomalonic ester are commercially available.

Bromomethyl 3-pyridyl ketone (I) as the hydrobromide or hydrochloride was condensed with sodioacetamidomalonic ester (II) to yield the desired 2-nicotinylmethyl 2-acetamidomalonic ester (III). The latter upon basic hydrolysis yielded γ-(3-pyridyl)-γ-oxo-α-acetamidobutyric acid (IV). The ammonium salt of this acid in the presence of methylamine, hydrogen, and Raney nickel afforded 3-acetamidocotinine (V). This acetamido compound was readily hydrolyzed to 3-aminocotinine (VI). The latter was diazotized in sulfuric acid to obtain hydroxycotinine (VII).

In the foregoing synthesis the steps leading from bromomethyl 3-pyridyl ketone through 3-aminocotinine are regarded as unequivocal, although degradations were not effected. In the diazotization of 3-aminocotinine to hydroxycotinine, 3-hydroxycotinine is considered the most likely product, although intramolecular rearrangements do accompany diazotization reactions. The assignment of the hydroxyl group to the 3-position of the pyrrolidone ring indicates, therefore, a probability and is not decisive in the absence of degradations and other corroborating investigations.

Aminocotinine (VI), which was not examined for possible diastereoisomeric forms, yielded upon diazotization hydroxycotinine which contained two isomeric pairs. These were separated by fractional crystallization. The high-melting form was converted to a chlorocotinine by reaction with thionyl chloride. Upon hydrogenolysis this chlorocotinine yielded (+, −)-cotinine, identified as the monopicrate in comparison with an authentic synthetic sample.

Resolution of the high-melting form of hydroxycotinine with tartaric acid led to the isolation of a dextrorotatory enantiomorph, m.p. 135–137°. The compound, which differed from metabolic (+)-hydroxycotinine in melting point, was cochromatographed on paper with the metabolic product in two solvent systems.[13]

The low-melting form of synthetic hydroxycotinine, upon resolution with tartaric acid, yielded a dextrorotatory product, m.p. 110–111°, $[\alpha]$ +47.30°. The melting point and R_f value agreed with that of the metabolic product. Upon admixture of the synthetic and natural product there was no melting-point depression. The close correspondence of the infrared absorption spectra[14] of the metabolic and effectively totally synthetic compounds provided further evidence for the identity of the natural and synthetic compounds.

Metabolic hydroxycotinine was converted to chlorocotinine by treatment with thionyl chloride. This chlorocotinine, upon hydrogenolysis, yielded (−)-cotinine which was characterized as the hydrobromide and compared with an authentic sample.

If it is considered that the hydroxyl group of metabolic hydroxycotinine is in position 3 (or 4) on the pyrrolidone ring—and position 3 appears likely on the basis of the synthetic method—the compound contains two asymmetric centers. The reduction of the chlorocotinine which was obtained from metabolic hydroxycotinine to (−)-cotinine would then indicate that in the metabolism of (−)-cotinine to hydroxycotinine the absolute configuration of the 5-carbon of the pyrrolidone ring of (−)-cotinine has been retained. Previous studies have shown[4] that this carbon atom of (−)-

(9) H. McKennis, Jr., L. B. Turnbull, S. L. Schwartz, E. Tamaki, and E. R. Bowman, *J. Biol.Chem.*, **237**, 541 (1962).

(10) H. B. Hucker and J. R. Gillette, *Federation Proc.*, **19**, 30 (1960).

(11) H. McKennis, Jr., L. B. Turnbull, H. N. Wingfield, Jr., and L. J. Dewey, *J. Am. Chem. Soc.*, **80**, 1634 (1958).

(12) In this connection it is interesting to note that R. H. Decker and R. R. Brown [*Federation Proc.*, **21**, 3 (1962)] have found that γ-(3-pyridyl)-γ-oxo-α-aminobutyric acid, derivable from one of the intermediates in our synthesis of hydroxycotinine, is an inhibitor of kynureninase and kynurenine hydroxylase.

(13) The compositions of the solvent systems which are designated as "acid" and "base" in this paper have been previously described (ref. 5).

(14) The authors are grateful to Mr. J. Scott Osborne of the Department of Research and Development, The American Tobacco Co., for these determinations.

cotinine has the same absolute configuration as that of the parent compound L-(−)-nicotine.[15]

Experimental

Bromomethyl 3-Pyridyl Ketone Hydrobromide.—To a solution of 3-acetylpyridine (2.5 g.) in 25 ml. of 48% hydrogen bromide in glacial acetic acid was added 7.2 g. of pyridine hydrobromide perbromide with stirring. The white crystalline product separated. Precipitation was completed by addition of ether. The salt was collected, washed with ether and was air-dried (5.5 g.; 92%). The product, which was sufficiently pure for condensation, was further purified by several recrystallizations from methanol–ether, m.p. 185–188° dec.

Anal. Calcd. for $C_7H_7NOBr_2$: C, 29.72; H, 2.51. Found: C, 29.79; H, 2.81.

The foregoing procedure appears to be more convenient than that employed by Wingfield,[16] who prepared the hydrobromide of bromomethyl 3-pyridyl ketone and presented analytical data, but no melting point.

A product, apparently a mixture of the hydrobromide and hydrochloride salts, which is also satisfactory for condensation reactions, was prepared from a solution of 3-acetylpyridine (45.6 g.) in 445 ml. of glacial acetic acid saturated with hydrochloric acid. Pyridine hydrobromide perbromide (130.5 g.) was added in one portion, and the mixture was mechanically stirred until precipitation of the colorless crystalline product was complete. The crystals were collected, washed once with methanol–ether (1–1 by vol.) and finally with ether. The air-dried product (87 g.), which was used directly or stored in diffuse light, was used interchangeably (on a weight basis) with the hydrobromide (above).

Ethyl γ-(3-Pyridyl)-γ-oxo-α-acetamido-α-carbethoxybutyrate.—To a solution of 233 g. of diethyl acetamidomalonate in 1 l. of absolute ethanol was added 27.7 g. of sodium chips in an atmosphere of nitrogen. After completion of the reaction under reflux, the solvent was removed under diminished pressure. The glassy residue, obtained by drying overnight at 1 mm., was pulverized and then added to a mixture of 1.5 l. of benzene and 145 g. of bromomethyl 3-pyridyl ketone hydrobromide. The mixture was refluxed with stirring for 48 hr. and then filtered. The benzene solution was extracted with 600 ml. of 0.1 *N* hydrochloric acid and then with 200 ml. of 0.1 *N* hydrochloric acid. Acetamidomalonic ester (92 g.) was recovered from the benzene layer. The combined aqueous layers were made alkaline with concentrated ammonia water (60 ml.) and then extracted with two portions of chloroform (500 ml. and 800 ml. each). The deeply colored chloroform layer was concentrated to 200 ml. and then placed on a column of acid-washed alumina (1 kg., Merck & Co., Rahway, N. J.). The column was eluted with 2 l. of methanol–ether (1–20 by vol.) and finally with 2 l. of methanol–ether (1–10 by vol.). The residue from evaporation of the combined eluates yielded, upon recrystallization from benzene–hexane, 105 g. of virtually colorless crystalline ethyl γ-(3-pyridyl)-γ-oxo-α-acetamido-α-carbethoxybutyrate, m.p. 126–128°. The product at this stage was sufficiently pure for hydrolysis and decarboxylation. For analysis a sample was recrystallized several times from benzene–hexane, m.p. 139.5–141.5°.

Anal. Calcd. for $C_{10}H_{20}N_2O_6$: C, 57.13; H, 5.99; N, 8.33. Found: C, 57.07; H, 5.78; N, 8.27.

Ammonium γ-(3-Pyridyl)-γ-oxo-α-acetoamidobutyrate.—Ethyl γ-(3-pyridyl)-γ-oxo-α-acetamido-α-carbethoxybutyrate (m.p. 126–128°, above, 210 g.) was stirred with 1272 ml. of 1.00 *N* sodium hydroxide at room temperature until solution was complete (approx. 2 hr.). Glacial acetic acid (110 ml.) was then added, and the solution was heated to boiling for 20 min. (evolution of carbon dioxide). The cooled solution was then placed upon a column of Dowex 50 (H^+), 2 l. The column was eluted with 2 *N* ammonia water until the eluate gave a negative Koenig reaction. The residue from evaporation of the ammoniacal solution was recrystallized from methanol to give 110 g. of colorless crystals, m.p. 181–182°. For analysis a sample was recrystallized from ethanol–acetone and air-dried, m.p. 181–183°.

Anal. Calcd. for $C_{11}H_{15}N_3O_4$: C, 52.16; H, 5.97; N, 16.59. Found: C, 52.18; H, 6.12; N, 16.7.

3-Acetamido-3-methylcarbamyl-1-methyl-5-(3′-pyridyl)pyrrolidone-2.—A solution of 3.0 g. of ethyl γ-(3-pyridyl)-γ-oxo-α-acetamido-α-carbethoxybutyrate in 40 ml. of methanol was hydrogenated at 32 atm. in the presence of 7.3 g. of methylamine and 1 g. of Raney nickel for 4 hr. at 85°. After removal of the catalyst and the solvent, the residue was recrystallized from methanol to obtain 2.3 g. of colorless crystals, which sintered at 137° and melted at 195.5°, λ_{max} 262 mμ, *k* 9.18 in methanol. The air-dried sample gave the correct analysis for a monohydrate, R_f 0.76 (base) and R_f 0.46 (acid).

Anal. Calcd. for $C_{14}H_{20}N_4O_4$: C, 54.53; H, 6.53; N, 18.17. Found: C, 54.60; H, 6.87; N, 18.1.

3-Acetamidocotinine.—A solution of 80 g. of ammonium γ-(3-pyridyl)-γ-oxo-α-acetamidobutyric acid (m.p. 181–182°) in 1200 ml. of absolute ethanol was hydrogenated at 80 atm. and 95° in the presence of 8 g. of Raney nickel and 260 g. of methylamine during 8 hr. After removal of the catalyst, the ethanolic solution was evaporated to dryness. The residue was dissolved in chloroform and placed upon a column of acid-washed alumina (1200 g.). Elution with methanol–chloroform (1–20 and 1–10 by vol.) served to remove material showing a single Koenig positive zone (R_f 0.62, base, and R_f 0.32, acid) upon paper chromatography. The residue from evaporation of the combined eluates, a straw-colored gum (48 g.), yielded a crystalline picrate upon treated with a saturated solution of picric acid (15% H_2O) in methanol. For analysis the yellow salt, 5-(3′-pyridyl)-3-acetamido-1-methylpyrrolidone-2 monopicrate, was recrystallized from aqueous methanol (m.p. 247–249°, dec.) and dried at 1 mm. and 50° for 2 hr.

Anal. Calcd. for $C_{18}H_{18}N_6O_9$: C, 46.76; H, 3.92. Found: C, 46.66; H, 3.70.

Hydroxycotinine (Mixed Isomers).—A solution of 144 g. of 3-acetamidocotinine (as the straw-colored gum obtained above) in 1085 ml. of 5 *N* hydrochloric acid was heated under reflux for 5 hr. During the period 3-acetamidocotinine (R_f 0.62, base) was hydrolyzed to aminocotinine (R_f 0.53, base, and R_f 0.17, acid). After cooling, the mixture was made alkaline with sodium hydroxide (216 g.) and then extracted with eight portions of chloroform (950 ml. each). Crude aminocotinine (86 g.) was obtained as a dark gum upon evaporation of the chloroform. Without further purification the aminocotinine was dissolved in 1 l. of 2 *N* sulfuric acid. Sodium nitrite (100 g.) was added slowly with stirring to the solution which was cooled in an ice–salt bath. When the addition was complete the solution was allowed to warm to room temperature and then made faintly alkaline to litmus by addition of sodium hydroxide pellets (approx. 80 g.). The solution was concentrated to 500 ml. under diminished pressure and then extracted with seven portions of chloroform (950 ml. each). The combined chloroform solutions upon evaporation yielded 53 g. of crude hydroxycotinine (A) as a dark viscous gum. The mixture showed upon paper chromatography Koenig positive zones at R_f 0.61 (base) and R_f 0.30 (acid), indicating completeness of diazotization. (A minor spot was occasionally observed at R_f 0.70, base.)

When the diazotization of 3-aminocotinine was conducted in hydrochloric acid rather than sulfuric acid it was possible to isolate a Koenig positive compound (R_f 0.76, base, and R_f 0.52, acid) which gave the correct analysis for 3-chlorocotinine. The chloroform solution obtained by extraction of the alkalinized diazotization mixture was dried over sodium sulfate and placed on a column of alumina. Elution with chloroform removed a halogen-containing component while hydroxycotinine remained on the column. The crystalline residue from evaporation of the chloroform was rechromatographed on alumina and eluted with methanol–ether to obtain a sample, m.p. 125–126°, which was recrystallized from acetone–hexane, m.p. 135–137°.

Anal. Calcd. for $C_{10}H_{11}N_2OCl$: C, 57.01; H, 5.26; N, 13.30. Found: C, 56.76; H, 5.43; N, 13.22.

Acetoxycotinine.—Crude hydroxycotinine (A) from the diazotization (8.3 g.) was treated with an excess of acetic anhydride in pyridine. After standing overnight the mixture was concentrated at the oil pump. The residue was dissolved in chloroform and placed on a column of acid-washed alumina. The column was eluted with ethanol–chloroform. The first fractions (from 2.5 to 5% methanol) upon evaporation yielded acetoxycotinine as a colorless oil, R_f 0.75 (base). The latter fractions (10% methanol) yielded a small amount of unesterified hydroxycotinine. The acetoxycotinine fraction was converted to a picrate with methanolic picric acid. The yellow salt was recrystallized several times from methanol, m.p. 167–169°, and then dried for 1 hr. at 50° and 1 mm.

(15) P. Karrer and R. Widmer, *Helv. Chim. Acta*, **8**, 364 (1925).

(16) Appreciation is expressed to Mr. H. N. Wingfield, Jr., for discussing this work with us prior to publication [*J. Org. Chem.*, **24**, 872 (1959)].

Anal. Calcd. for $C_{18}H_{17}N_5O_{10}$: C, 46.66; H, 3.70; N, 15.12. Found: C, 46.66; H, 3.35; N, 15.15, 15.3.

(+, −)-Hydroxycotinine, Isomer I.—A sample of the foregoing acetoxycotinine picrate was dissolved in water and placed upon a column of Dowex 50 (H^+). After a water wash to remove picric acid the column was eluted with 0.1 *N* ammonia water until no more Koenig positive material could be obtained. The solution showed upon paper chromatography a single Koenig positive zone (R_f 0.61, base; R_f 0.30, acid). The solution was extracted with chloroform to give a light tan oil which solidified after standing approximately 1 week. The resultant hydroxycotinine was recrystallized from acetone, m.p. 149.5–151°, and dried at 1 mm. and 50°. The same product was also obtained directly from the crude hydroxycotinine (A from the diazotization above). A solution of 31 g. of A in 60 ml. of acetone upon cooling deposited 21 g. of almost colorless crystals, m.p. 127–139°. The solution, after concentration to 30 ml. and upon cooling, deposited an additional crop, 2.2 g., m.p. 99–113°. The mother liquor (B) yielded another isomeric pair as described below. The combined crops were recrystallized five times from methyl ethyl ketone to give 9.0 g., m.p. 146–148°. After several recrystallizations from acetone the product, which was dried at 50° and 1 mm., melted at 149–151°.

Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C, 62.48; H, 6.29; N, 14.57. Found: C, 62.2; H, 6.48; N, 14.25.

(+)-Hydroxycotinine, High-melting Isomer.—(+, −)-Hydroxycotinine, m.p. 149.5–151°, (5 g.) and 5 g. of (+)-tartaric acid were dissolved in 28 ml. of methanol. Upon cooling and scratching, a precipitate formed. Two recrystallizations from methanol afforded 3 g. of a tartrate, m.p. 159–161°. The salt was dissolved in 20 ml. of 5 *N* ammonia water, and the solution was extracted with five portions of chloroform (25 ml. each). The oily residue from evaporation of the chloroform crystallized on standing and weighed 890 mg. after two recrystallizations from acetone. The air-dried material melted at 86–128° and formed crystals, m.p. 135–137°, upon drying overnight at 50° and 1 mm.

Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C, 62.48; H, 6.29; N, 14.57. Found: C, 62.25; H, 6.39; N, 14.40. $[\alpha]^{26}_{5461}$ +46.2° (*c*, 10 in methanol).

(+, −)-Hydroxycotinine, Isomer II.—The mother liquor (B) from the high-melting isomer (above) was cooled in the refrigerator to give three crops (3.0 g., m.p. 71–72°; 1.1 g., m.p. 70–71.5°; 0.7 g., m.p. 70–71°) and finally 3.0 g. of gum. The combined crystalline crops were recrystallized four times from benzene to give an analytical sample melting at 70.5–72° after drying overnight at 25° and 1 mm.

Anal. Calcd. for $C_{10}H_{12}N_2O_2 \cdot {}^1/_2\ H_2O$: C, 59.69; H, 6.51; N, 13.92. Found: C, 59.80, 59.52; H, 6.34, 6.42; N, 13.72, 13.79.

Tartaric Acid Salt of Metabolic (+)-Hydroxycotinine.—A solution containing 44 mg. of metabolic (+)-hydroxycotinine, m.p. 109.5–110.5°, obtained from the metabolism of (−)-cotinine in the human[7] and 36 mg. of (−)-tartaric acid in 0.5 ml. of methanol was treated with ether until a faint turbidity was produced and then was allowed to stand for several hours while a tartrate of (−)-hydroxycotinine precipitated. The salt, which was suitable for seeding, melted at 148–150°.

(+)-Hydroxycotinine, Low-melting Isomer.—A solution of 2.0 g. of (+,−)-hydroxycotinine, m.p. 70.5–72°, and 1.49 g. of (−)-tartaric acid in 10 ml. of methanol was diluted slowly with ether (approx. 5 ml.) until a slight turbidity persisted. The cloudy solution was seeded with a small crystal of the tartrate of metabolic hydroxycotinine (above). Scratching was continued until a crystalline fraction began to appear. The mixture was then allowed to stand overnight. The crystalline tartrate was collected and crystallized twice from methanol–ether, 750 mg., m.p. 140–142°. The salt was dissolved in ammonia water and extracted with chloroform as described for the resolution of the high-melting isomer (above). The residue from evaporation of the chloroform, m.p. 95–103°, was crystallized twice from ether–acetone to give 125 mg. of (+)-hydroxycotinine, m.p. 110–111°, which was dried for analysis at 28° and 1 mm.

Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C, 62.48; H, 6.29; N, 14.57. Found: C, 62.56; H, 6.36; N, 14.70. $[\alpha]^{32.5}D$ +47.3° (*c*, 5.5 in methanol).

Upon admixture with a sample of metabolic hydroxycotinine,[7] m.p. 110–111°, $[\alpha]^{29}D$ +49.2°, the melting point of the foregoing synthetic (+)-hydroxycotinine showed no depression.

(+)-Hydroxycotinine picrate.—A sample of synthetic (+)-hydroxycotinine, m.p. 110–111°, was treated with methanolic picric acid. The yellow precipitate was recrystallized from methanol, m.p. 134.5–135.5°. The sample for analysis, which was dried at 25° and 1 mm., did not depress the melting point of the picrate of metabolic hydroxycotinine,[7] m.p. 134–136°.

Anal. Calcd. for $C_{16}H_{15}N_5O_9$: C, 45.61; H, 3.58; N, 16.62. Found: C, 45.78; H, 3.51; N, 16.61.

(+,−)-Cotinine.—A solution of 450 mg. of γ-(3-pyridyl)-γ-oxobutyric acid and 5 g. of methylamine was hydrogenated at 1 atm. and room temperature in the presence of 300 mg. of platinum on barium sulfate until approximately 1 equivalent of hydrogen had been taken up. After removal of the catalyst, the solution was evaporated to a clear gum (484 mg.). Upon paper chromatography in acidic system, the mixture showed a Koenig positive spot at R_f 0.27, corresponding in R_f value to authentic γ-(3-pyridyl)-γ-methylaminobutyric acid and another zone at R_f 0.33 corresponding to γ-(3-pyridyl)-γ-hydroxybutyric acid (obtained as a crude product from hydrogenation of γ-(3-pyridyl)-γ-oxobutyric acid as above but in the absence of methylamine). The gum was heated to 150–160° in an atmosphere of nitrogen for 10 min. The product was treated with chloroform–acetone (1–1 by vol.). An insoluble substance (79 mg.), R_f 0.33 (acid) was separated by filtration. The solution was placed upon a column of Florisil. An elution with acetone yielded an initial fraction which upon paper chromatography in the acid system showed a Koenig positive zone at R_f 0.38 and a subsequent fraction with Koenig positive material at R_f 0.38 and 0.42. The first fraction upon evaporation yielded 63 mg. of (+, −)-cotinine as an oil. Upon treatment with alcoholic picric acid the oil formed a yellow picrate which was recrystallized from methanol, m.p. 127–129°.

Anal. Calcd. for $C_{16}H_{15}N_5O_8$: C, 47.41; H, 3.73; N, 17.28. Found: C, 47.27; H, 3.96; N, 17.50.

(+,−)-Chlorocotinine.—(+,−)-Hydroxycotinine (isomer I), 340 mg., and 6 ml. of thionyl chloride were heated under reflux for 2 hr. The product, obtained by evaporation of the solvent, was dissolved in water and then placed on a column of Dowex 50 (H^+). The column was eluted with 0.1 *N* ammonia water, and the eluate was evaporated to obtain a crystalline residue, m.p. 115–123°. The analytical sample (305 mg.) was obtained by several recrystallizations from hexane–acetone, m.p. 132–135°, after drying at room temperature and 1 mm.

Anal. Calcd. for $C_{10}H_{11}N_2OCl$: C, 57.01; H, 5.26; N, 13.30. Found: C, 57.23; H, 5.04; N, 13.85.

(+,−)-Cotinine from (+,−)-Chlorocotinine.—A solution of 250 mg. of (+,−)-chlorocotinine (above) in 30 ml. of 95% ethanol containing 0.5 ml. of concentrated ammonia water was hydrogenated at atmospheric pressure in the presence of 300 mg. of 5% palladium on charcoal for 0.5 hr. After removal of the catalyst, the solvent was evaporated to obtain an oily residue (267 mg.), which cochromatographed with authentic (−)-cotinine in both the acidic and basic systems. The oil was dissolved in chloroform and placed on a column of Florisil. The column was eluted with acetone. The clear gummy residue (150 mg.) obtained from evaporation of the acetone was treated with an equivalent of picric acid (10% water) as a saturated solution in ethanol. The yellow crystalline picrate was recrystallized from ethanol and, after drying at room temperature and 1 mm., melted at 127–129°. The mixed melting point with an authentic sample of (+,−)-cotinine monopicrate (above) showed no depression.

Anal. Calcd. for $C_{16}H_{15}N_5O_8$: C, 47.41; H, 3.73; N, 17.28. Found: C, 46.93; H, 3.81; N, 17.35.

Chlorocotinine from Metabolic (+)-Hydroxycotinine.—A solution of 150 mg. of metabolic (+)-hydroxycotinine in 10 ml. of thionyl chloride was heated under reflux for 2 hr. The residue (153 mg.) from evaporation of the solvent was dissolved in water and placed upon a column (1 × 5 cm.) of Dowex 50 (H^+). After a water wash, the column was eluted with 0.1 *N* ammonia water. The residue from evaporation of the eluate was dissolved in 35–60° petroleum ether–acetone (1–1 by vol.). The cooled solution deposited colorless crystals, m.p. 108–110°, which showed a single Koenig positive zone at R_f 0.54 (acid) and R_f 0.74 (base). For analysis the sample was recrystallized from petroleum ether–acetone to a constant melting point of 112–114° which was unchanged upon drying at 60° over potassium hydroxide and 1 mm. for 8 hr.

Anal. Calcd. for $C_{10}H_{11}N_2OCl$: C, 57.01; H, 5.26; N, 13.30. Found: C, 57.20; H, 5.26; N, 13.10.

(−)-Cotinine from Metabolic (+)-Hydroxycotinine.—A solution of 645 mg. of chlorocotinine (obtained from metabolic (+)-hydroxycotinine) in 75 ml. of ethanol was hydrogenated at atmospheric pressure and room temperature in the presence of 350 mg. of 5% palladium on charcoal until 1 equivalent of hydrogen had been consumed. After removal of the catalyst, the solution was evaporated to dryness. A solution of the residue in ammonia water was extracted with chloroform. The chloroform solution was dried over sodium sulfate and then placed upon a column of alumina. The column was eluted with methanol to obtain 420 mg. of (−)-cotinine. To this was added 1 equivalent of picric acid in methanol. The crystalline monopicrate melted at 105–106° after recrystallization from methanol. The picrate was decomposed in dilute hydrochloric acid. After extraction of picric acid with ethyl acetate, the aqueous solution was adjusted to pH 10 with ammonia water and extracted with chloroform. The cotinine base from evaporation of the chloroform was treated with one equivalent of hydrobromic acid to give (−)-cotinine hydrobromide. The analytical sample was recrystallized from isopropyl alcohol and dried at 25° and 1 mm., m.p. 187–188° dec. $[\alpha]^{30}_{6461}$ −32.6°, (*c*, 7.4 in methanol).

Anal. Calcd. for $C_{10}H_{13}N_2OBr$: C, 46.71; H, 5.09; N, 10.90. Found: C, 46.65; H, 4.96; N, 10.92.

The foregoing hydrobromide had a melting point and optical rotation in substantial agreement with the samples prepared[7] from metabolic and synthetic (−)-cotinine.

Isolation of Hydroxycotinine from Smokers' Urine.—Smoker's urine (60 l.) was obtained as voluntary daytime contributions from male laboratory workers, made alkaline, and then continuously extracted with chloroform as previously described.[7] The chloroform solution upon evaporation yielded a dark-brown oily residue (7.0 g.). The residue was treated with 40 ml. of boiling water, and the cooled mixture was filtered. The filtrate was adjusted to pH 2 with 5 *N* hydrochloric acid. The acidic solution was placed on a column of (4 × 30 cm.) of Dowex 50 (H^+). The column was washed thoroughly with water. Koenig positive material of R_f values 0.34, 0.61, 0.73, and 0.90 (base) was removed by exhaustive elution with 0.1 *N* ammonia water. The ammonical solution was placed upon a column (4 × 30 cm.) of Dowex 21K (OH^-). The ammoniacal effluent and exhaustive water wash were combined and concentrated to an oily residue (1.1 g.). The residue was dissolved in 20 ml. of chloroform and placed on a column of acid-washed alumina (30 g.). An elution with ether containing successively increasing amounts of methanol (0–100% by vol.) served to remove a fraction with Koenig positive material, R_f 0.75 (base), which was identified as cotinine. Subsequent fractions contained material with R_f 0.61 (base) and the final fractions contained material showing a single Koenig positive zone at R_f 0.84 (base), which was cochromatographed with nicotine but was not identified as such. The combined R_f 0.61 fractions yielded upon evaporation an oily residue (118 mg.). The residue was treated with 1 ml. of dry pyridine and 1 ml. of acetic anhydride. After standing overnight at room temperature, the mixture was concentrated under diminished pressure. The oily residue (143 mg.) was dissolved in 5 ml. of chloroform and then placed upon a column of acid-washed alumina (5 g.). Elution with ether containing increasing amounts of methanol (0–100%) gave fraction A, which showed a single Koenig positive spot upon paper chromatography (R_f 0.75, base), and fraction B (R_f 0.61, base). The oil (17 mg.) from fraction B cochromatographed on paper with authentic crystalline (−)-demethylcotinine[6] in both acid and base. It failed to crystallize and was not identified. Fraction A was obtained as an oil (30 mg.). A sample (15 mg.) in 0.5 ml. of ethanol was treated with one equivalent of picric acid (15% water) as a saturated solution in ethanol. The resultant crystalline acetoxycotinine picrate (75 mg.) was recrystallized from ethanol, m.p. 165°. The analytical sample corresponded in melting point[17] to acetoxycotinine picrate which was obtained from studies on the dog.[5] Admixture produced no depression of the melting point. The two samples cochromatographed, R_f 0.77 (acid) and R_f 0.74 (base). For analysis the compound from smokers' urine was dried at 60° and 1 mm. over potassium hydroxide.

Anal. Calcd. for $C_{18}H_{17}N_5O_{10}$: C, 46.67; H, 3.70; N, 15.12. Found: C, 46.62; H, 3.74; N, 15.19.

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(17) This corrected capillary melting point was obtained at a heating rate of 0.30° per min. At approximately 140° a change in crystalline form was observed. At higher rates of heating the melting point is somewhat elevated (ref. 5).

A Comparison of Methods for the Preparation of 2- and 4-Styrylpyridines[1]

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Derivatives of 2-styrylpyridine, 5-ethyl-2-styrylpyridine, and 4-styrylpyridine bearing CH_3O, NO_2, CH_3, and $N(CH_3)_2$ in the 4′-position have been prepared by the following routes: (1) pyrolysis of the corresponding methiodides; (2) reaction of a benzaldehyde with a picoline in refluxing acetic anhydride; and (3) the zinc chloride condensation of a benzaldehyde and a picoline at 200°. A (2:5) mixture of *cis*- and *trans*-2-styrylpyridine was obtained from the reaction between a phosphorus ylid and 2-pyridylaldehyde. An analogous preparation gave *cis*-4-styrylpyridine from 4-pyridylaldehyde.

In preceding papers,[2] it was shown that the reaction of benzaldehyde with 2-picoline in refluxing acetic anhydride gave *trans*-2-styrylpyridine. Irradiation of *trans*-2-styrylpyridine, its hydrochloride or methiodide in solution with ultraviolet light gave the *cis* modification. In order to extend this study to include substituted and structurally isomeric styrylpyridines, it was necessary to examine various preparative methods for convenience, yields, and the isomer configurations obtained by these procedures.

Four methods were employed for the preparation of the styrylpyridines and are described in order as follows.

Method 1.—Phillips[3] prepared styrylpyridine methiodides by condensation of benzaldehydes with 2-picoline methiodide in methanol solution using piperidine as the catalyst. Horwitz[4] showed that the *trans* salts resulted when the same reaction was conducted using quinaldine methiodides in place of 2-picoline methiodide. On the basis of previous spectroscopic results from these laboratories,[2b] we have assigned the *trans* configuration to styrylpyridine methiodides prepared using piperidine as the catalyst. The physical properties of the styryl-

(1) Communication no. 2312 from the Kodak Research Laboratories, Eastman Kodak Co., Rochester, N. Y.

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