The Synthesis of Anatabine and Related Compounds[1]

P. M. QUAN, T. K. B. KARNS, AND LOUIS D. QUIN[2]

Department of Chemistry, Duke University, Durham, North Carolina

Received March 1, 1965

Boron trifluoride is known to catalyze a reaction between aldehyde bis(carbamates) and substituted 1,3-butadienes leading to 1-ethoxycarbonyl-1,2,3,6-tetrahydropyridines. Under normal conditions butadiene itself will not take part in this reaction, which also appears to fail with bis(carbamates) derived from aldehydes of the pyridine series. It is shown that both these disadvantages may be overcome by use of a large excess of catalyst and a suitable solvent. The 1-ethoxycarbonyl-1,2,3,6-tetrahydropyridines are valuable intermediates which can be hydrolyzed to the free bases or directly reduced to 1-methyl-1,2,3,6-tetrahydropyridines. In this way syntheses of the alkaloids *dl*-anatabine and *dl*-N-methylanatabine and of several of their derivatives have been accomplished. N.m.r. data for these compounds fully confirm the assigned structures.

l-Anatabine (IVa) has been recognized for many years as one of the minor alkaloids of tobacco. It was first isolated[3a] from *Nicotiana tabacum* L., and has been detected in *N. glutinosa*[4] as well as in numerous tobacco varieties.[5] It is reported to be the most abundant of the minor alkaloids in the roots of these plants,[4] although it is present also in the leaf and stem. Also isolated from tobacco were racemic anatabine, which was resolved,[3b] and *l*-N-methylanatabine (Va) which was synthesized from *l*-anatabine.[3c] The absolute configurations of these alkaloids have been recently deduced.[6] The survival of anatabine in cigar[7] and cigarette[8] smoke has been demonstrated, but it has remained unavailable in sufficient quantity for pharmacological testing, and the structure assigned to it has not hitherto been confirmed by synthesis.

Hydrogenation and dehydrogenation studies by Späth and Kesztler[3a] showed anatabine to be a 2-(3′-pyridyl)tetrahydropyridine. As N-benzoylanatabine gave hippuric acid on oxidation, the isolated double bond was assigned to the 4,5-position, formulating the alkaloid[3a,4] as 1,2,3,6-tetrahydro-2,3′-bipyridine (IVa). The position of the isolated double bond in anatabine has been queried by a reviewer,[9] although no further evidence was presented. We describe the first total syntheses of *dl*-anatabine and *dl*-N-methylanatabine, confirming the structures IVa and Va. The same route was used to make the 4-chloro and 4,5-dimethyl derivatives of anatabine.

The Diels–Alder synthesis seemed attractive as a method of forming directly the tetrahydropyridine ring with the double bond in the 4,5-position. However, in a model experiment benzylidenebenzylamine failed to react with isoprene. A recent communication[10] described the synthesis of 2-aryl-1-ethoxycarbonyl-1,2,3,6-tetrahydropyridines by a not unrelated process, which involved the boron trifluoride catalyzed condensation of substituted 1,3-butadienes with the bis(carbamates) of aryl aldehydes. The N-ethoxycarbonyl group was removable by hydrolysis. These workers reported little success with butadiene itself, nor were examples of the use of heterocyclic aldehydes given, although the bis(carbamate) of 2-formylpyridine was described. We found that a typical pyridine derivative could be made to react only in the presence of a massive excess of catalyst. Thus, in monoglyme solution, 2,3-dimethyl-1,3-butadiene reacted with the bis(carbamate) of 3-formylpyridine (I) in the presence of 1500 mole % of the catalyst to provide an optimum yield of 55% of 1-ethoxycarbonyl-1,2,3,6-tetrahydro-4,5-dimethyl-2,3′-bipyridine (IIc, see Scheme I). The bis(acetamide) of 3-formylpyridine was also tried in this reaction, but proved less reactive than the bis(carbamate). Initial results with butadiene were less satisfactory. With monoglyme as solvent we could obtain at best an 8% yield of the N-ethoxycarbonyl compound IIa from I. When the solvent was changed to glacial acetic acid and boron trifluoride–bis(acetic acid) was used as the catalyst, this yield was raised to 20%. These conditions were also used in the synthesis of the 4-chloro derivative IIb from 2-chloro-1,3-butadiene and I. The low yield in this reaction (28%) may be due to the rapidity with which the product polymerized; a transparent skin formed within a few minutes on the surface of the liquid after distillation, necessitating its storage in solution.

From the reactions in which boron trifluoride etherate was used as catalyst, a side product was isolated by chromatography on alumina in yields up to 19%. From the infrared spectrum, which showed carbonyl and –NH– groups, and the n.m.r. spectrum, which showed 3-pyridyl and ethoxy groups, this was considered to be ethyl N-(3-pyridylmethyl)carbamate (III). As this was a somewhat unexpected side product, the structure was confirmed by unambiguous synthesis of III from 3-picolylamine and ethyl chloroformate. The mechanism by which this compound arose is under investigation.

The n.m.r. spectra[11] of the intermediates (IIa, b, c) showed characteristic 3-pyridyl and ethoxy resonances, and two, one, and zero olefinic protons, respectively.

(1) Preliminary communication: P. M. Quan, T. K. B. Karns, and L. D. Quin, *Chem. Ind.* (London), 1553 (1964).

(2) To whom enquiries should be addressed.

(3) E. Späth and F. Kesztler, (a) *Ber.*, **70**, 239 (1937); (b) *ibid.*, **70**, 704 (1937); (c) *ibid.*, **70**, 2450 (1937).

(4) E. Wada, T. Kisaki, and M. Ihida, *Arch. Biochem. Biophys.*, **80**, 258 (1959).

(5) H. Kuhn and H. Bühn, *Fachliche Mitt. Oesterr. Tabakregie*, 6 (1956).

(6) (a) I. Ribas-Marques and A. N. Blanco, *Anales real soc. espan. fis. quim.* (Madrid), **B57**, No. 12, 781 (1961); (b) R. Lukes, A. A. Arojan, J. Kovar, and K. Blaha, *Collection Czech. Chem. Commun.*, **27**, 751 (1962).

(7) F. Kuffner, K. Schick, and H. Bühn, *Monatsh. Chem.*, **87**, 749 (1956).

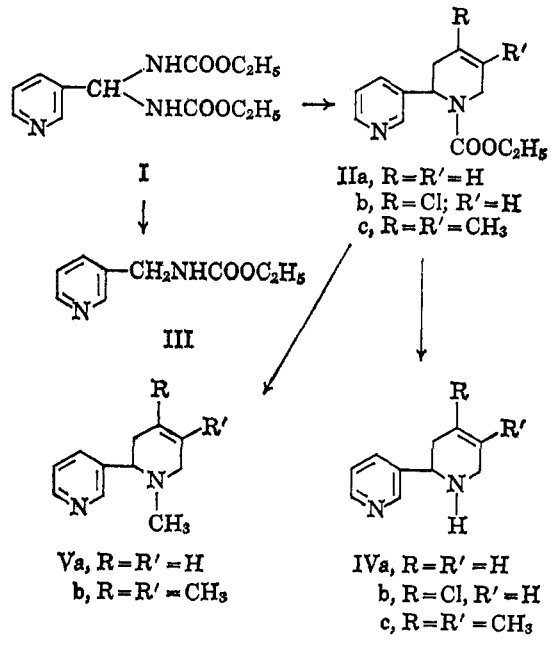
(8) L. D. Quin, *J. Org. Chem.*, **24**, 911, 914 (1959).

(9) K. E. Jackson, *Chem. Rev.*, **29**, 123 (1941).

(10) R. Merten and G. Müller, *Angew. Chem.*, **74**, 866 (1962).

(11) N.m.r. spectra were measured on deuteriochloroform solutions with a Varian A-60 n.m.r. spectrometer. Tetramethylsilane (δ 0.0) was used as an internal standard.

SCHEME I



Hydrolysis of IIa and IIb to the corresponding bases *dl*-anatabine (IVa) and its 4-chloro derivative (IVb) proceeded satisfactorily in aqueous–alcoholic potassium hydroxide at 110–125°; a slightly higher temperature was required for hydrolysis of the dimethyl compound IIc. At the higher temperatures used in the corresponding benzene series,[10] considerable decomposition of these ring systems occurred.

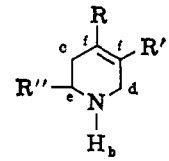
Reduction of IIa and IIc with lithium aluminum hydride gave, respectively, *dl*-N-methylanatabine (Va) and its 4,5-dimethyl derivative, Vb. We were unable to obtain a sample of natural *l*-N-methylanatabine for comparison with the synthetic product. However, the structure of the former is not in doubt as it has been synthesized from *l*-anatabine.[3c] Catalytic dehydrogenation of anatabine[3a,4] gave 2,3′-bipyridine, identified by its infrared spectrum[12] and by preparation of the dipicrate.[12]

The nonaromatic proton resonances of the bases IV[12a] are compared in Table I with the published[13] spectrum of 1,2,3,6-tetrahydropyridine (VI).

The spectra of the family of compounds resemble each other closely; with the exception of the anticipated downfield shift of the e-protons caused by the pyridyl substituent, similar chemical shifts for comparable protons are noted. The 4,5-position of the double bond, as expected from the synthetic method used, is supported by two observations. First, the small shift difference (0.03 p.p.m.) between the two f-protons in IVa, and the identity of the two methyl groups (a) in IVc exclude the 5,6-position for the double bond; the 5- and 6-positions in IVa would be quite dissimilar in this case, as would the 4- and 5-positions in IVc. Second, in all four compounds the e-proton appears as a triplet (J = 7 c.p.s.); it is thus implicated in coupling with the two c-protons which appear as a doublet[14] (J = 7 c.p.s.). Since there are two c-protons, the double bond cannot be in the 3,4-position.

TABLE I

PROTON CHEMICAL SHIFTS IN TETRAHYDROPYRIDINES[11]



VI, R = R′ = R″ = H
IVa, R = R′ = H; R″ = C_5H_4N
IVb, R = Cl; R′ = H; R″ = C_5H_4N
IVc, R = R′ = CH_3; R″ = C_5H_4N

	Chemical shifts, p.p.m. from TMS					
Compd.	a[a]	b[b]	c	d	e	f
VI	...	1.63	2.07	3.33	2.95	5.72 and 5.77[c]
IVa	...	2.17	2.20	3.50	3.85	5.77 and 5.80[c]
IVb	...	2.37	2.42	3.54	3.94	5.91
IVc	1.60	1.89	2.10	3.29	3.77	...

[a] Refers to the methyl protons when R = R′ = CH_3. [b] These shifts were particularly dependent on concentration. [c] Two other faint lines appear giving an AB spectrum. In IVa these are at 5.58 and 5.99 p.p.m., from which J_{AB} = 11.4 c.p.s. and $\Delta\nu$ = 6.7 c.p.s.

From a 2-substituted diene it is possible to produce tetrahydropyridines with a substituent in either the 4- or 5-position. Whereas isoprene gave rise to both such isomers, Merten and Müller found[10] that chloroprene gave only one isomer, claimed to be the 4-chloro derivative although the evidence for this was not included. The n.m.r. spectrum of IVb is in accord with this assignment. On comparing IVb with IVa it is seen that a greater downfield shift in the c-protons than the d-protons has occurred. This suggests that the c-protons are being deshielded by the presence of chlorine on an adjacent carbon atom. We were unable to detect any 5-chloro isomer by gas chromatography of either the N-ethoxycarbonyl compound IIb or its hydrolysis product IVb.

The infrared spectrum of IVa was identical with that published for naturally occurring anatabine.[4] The identity was further confirmed by gas and paper chromatography of the natural and synthetic bases in several systems,[4,7] and by preparation of the dipicrate, m.p. 198–200° (lit.[3b] m.p. 201–201.5°).

Although the yields in this synthesis are not high, it should prove convenient for compounds of the anabasine series, and as an unambiguous route to bipyridines, since the tetrahydropyridine ring in anatabine is readily reduced or catalytically dehydrogenated.[3a,4]

Experimental

General.—Melting points were determined in capillary tubes (evacuated and sealed in the case of dipicrates) with a Mel-Temp apparatus and are corrected. Infrared spectra were measured, unless otherwise stated, on liquid films with a Perkin-Elmer Model 21 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by Triangle Chemical Laboratories, Inc., Chapel Hill, N. C. For gas chromatography a Perkin-Elmer Model 154-B Vapor Fractometer,

(12) I. Onishi and K. Yamasaki, *Bull. Agr. Chem. Soc. Japan*, **21**, 177 (1957).

(12a) NOTE ADDED IN PROOF.—Our attention has been drawn to a published n.m.r. spectrum of anatabine, which shows excellent agreement with our synthetic material: I. Yamamoto, H. Kamimura, and R. Yamamoto, *Mem. Tokyo Univ. Agr.*, **7**, 67 (1963).

(13) N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "N.M.R. Spectra Catalog," Vol. 1, Varian Associates, Palo Alto, Calif., 1963, Spectrum No. 115.

(14) In solution, further splitting of the c-protons was observed with IVa and IVb. To observe the multiplicity of c in the case of IVa it was necessary to use the pure liquid, when the interfering proton b was shifted downfield. Under these conditions c appeared as a broad doublet.

employing helium as carrier gas, was used with either of two columns: column 1, 100 × 0.3 cm., 15% Dow-Corning silicone oil 710 on 60–80-mesh Chromosorb W; column 2, 200 × 0.3 cm., 20% polypropylene glycol on 60–80-mesh Chromosorb P.

Diethyl N,N'-(3-Pyridylmethylene)bis(carbamate) (I).—A solution of 3-formylpyridine (29.2 g., 0.27 mole), ethyl carbamate (48.6 g., 0.55 mole), and *p*-toluenesulfonic acid (0.35 g.) in 300 ml. of benzene was heated under reflux for 5 days below a Dean–Stark trap (preferably this reaction was conducted in an atmosphere of nitrogen). The mixture was allowed to cool, and the precipitated product was separated by filtration and recrystallized from benzene containing a little ethanol to give I (58 g., 80%) as colorless needles, m.p. 164–165.5°.

Anal. Calcd. for $C_{12}H_{17}N_3O_4$: C, 53.92; H, 6.41; N, 15.72. Found: C, 54.08; H, 6.33; N, 15.49.

N,N'-(3-Pyridylmethylene)bis(acetamide).—Reaction of 3-formylpyridine with 2 moles of acetamide by the above procedure gave N,N'-(3-pyridylmethylene)bis(acetamide), m.p. 239–240° (85%).

Anal. Calcd. for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32. Found: C, 57.69; H, 6.25.

Reaction of I with 2,3-Dimethyl-1,3-butadiene.—The procedure described gives a higher yield than that previously reported.[1] Boron trifluoride etherate was distilled before use.[15] A suspension of I (53.4 g., 0.200 mole) in 240 ml. of monoglyme was warmed to 60° in a flask fitted with reflux condenser, dropping funnel, thermometer, and mechanical stirrer. Boron trifluoride etherate (420 g., 3.0 mole) was cautiously run in, and the mixture was stirred for 3 hr. During this time the complex that precipitated on addition of the catalyst redissolved to form a deep red solution. The temperature was maintained at 60° while a solution of 2,3-dimethyl-1,3-butadiene (24.6 g., 0.280 mole) in 60 ml. of monoglyme was added during 3 hr. After a further 5 hr., the solution was allowed to cool, left to stand overnight, and then cautiously poured into excess aqueous sodium carbonate. This mixture was extracted with three 500-ml. quantities of ether; the ether solutions were combined, back-washed with aqueous sodium carbonate, and dried with magnesium sulfate. The solvent was distilled, leaving 60.4 g. of residual oil which was distilled through a short column, the fraction of b.p. 131–133° at 0.25 mm. (31.2 g.) being collected. This material was dissolved in excess 10% hydrochloric acid and shaken with ether, extracting 2.3 g. of nonbasic material. The acid solution was made basic with sodium carbonate and extracted several times with ether. The ether solutions were combined and dried with magnesium sulfate, and the ether was distilled, leaving 28.8 g. (55%) of **1-ethoxycarbonyl-1,2,3,6-tetrahydro-4,5-dimethyl-2,3'-bipyridine (IIc.)** This material, a pale yellow oil, showed only one peak on gas chromatography (column 1, 200°). The analysis sample, prepared by careful redistillation (b.p. 144–145° at 0.50 mm.), was almost colorless. The infrared spectrum showed carbonyl (1700 cm.$^{-1}$) and pyridyl (1590, 770, and 713 cm.$^{-1}$) absorptions and the absence of –NH– linkages.

Anal. Calcd. for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76. Found: C, 69.17; H, 7.81; N, 10.83.

A **picrate**, m.p. 161–162°, was prepared in, and recrystallized from, ethanol solution.

Anal. Calcd. for $C_{21}H_{23}N_5O_9$: C, 51.54; H, 4.74; N, 14.31. Found: C, 51.83; H, 4.68; N, 14.24.

Reaction of I with 1,3-Butadiene.—Boron trifluoride–bis(acetic acid) complex (240 g., 1.3 mole) and 1,3-butadiene (30 g., 0.55 mole) were added to a solution of I (49.7 g., 0.186 mole) in 210 ml. of glacial acetic acid in a 2-l. stainless steel autoclave and heated for 15 hr. at 80°. The autoclave was allowed to cool and the contents were slowly run into a solution of sodium carbonate (700 g.) in water (3 l.). The brown tar extracted by ether from this mixture (61 g.) was placed on a column of alumina (200 g., Fisher Scientific Co.). Elution with pentane removed hydrocarbon material, presumably formed by polymerization of 1,3-butadiene. A pentane–ether (3:1) mixture then eluted 8.6 g. (20%) of **1-ethoxycarbonyl-1,2,3,6-tetrahydro-2,3'-bipyridine (IIa)**, b.p. 125–128° at 0.3 mm. The infrared spectrum was very similar to that of IIc.

Anal. Calcd. for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.39; H, 7.07; N, 11.89.

Reaction of I with 2-Chloro-1,3-butadiene.—2-Chloro-1,3-butadiene was distilled shortly before use into a receiver containing a few crystals of 1,3,5-trinitrobenzene as a polymerization inhibitor.[16] Boron trifluoride–bis(acetic acid) complex (129 g., 0.69 mole) was added to a solution of I (36.7 g., 0.137 mole) in 165 ml. of glacial acetic acid. The temperature was raised to 60°, and a solution of 2-chloro-1,3-butadiene (18.2 g., 0.205 mole) in 65 ml. of glacial acetic acid was added during 1.5 hr. The temperature was kept at 60° for 12 hr.; the mixture was then allowed to cool and the crude product was isolated and chromatographed on alumina as described above. Distillation of the pentane–ether (1:1) eluate gave 10.4 g. (28%) of **4-chloro-1-ethoxycarbonyl-1,2,3,6-tetrahydro-2,3'-bipyridine (IIb)**, b.p. 165–167° at 0.4 mm. As this compound rapidly polymerized, the infrared and n.m.r. spectra were measured immediately, and the bulk of the material was dissolved and stored in ethanol solution. An aliquot of this solution was used to prepare the **picrate**, which was recrystallized from water and then from ethanol, m.p. 119–121°. The infrared spectrum showed carbonyl (1700 cm.$^{-1}$) and pyridyl (1580, 772, and 715 cm.$^{-1}$) absorptions.

Anal. Calcd. for $C_{19}H_{18}ClN_5O_9$: C, 46.03; H, 3.66; N, 14.12. Found: C, 46.13; H, 3.55; N, 13.95.

Isolation of the Side Product III.—A solution of the bis(carbamate) I (13.35 g., 0.05 mole) in 60 ml. of monoglyme and boron trifluoride etherate (106 g., 0.75 mole) was treated with a solution of 2-chloro-1,3-butadiene (6.64 g., 0.075 mole) in 20 ml. of monoglyme; the reaction procedure and work-up of the crude product were as described above for 2,3-dimethyl-1,3-butadiene. The crude product was distilled, and the total distillate (2.7 g., b.p. 50–180° at 0.5 mm.) was chromatographed on alumina (80 g.). Ether eluted 4-chloro-1-ethoxycarbonyl-1,2,3,6-tetrahydro-2,3'-bipyridine (IIb, 1.09 g., 8%); elution with ethyl acetate then gave **ethyl N-(3-pyridylmethyl)carbamate (III**, 1.12 g., 12%), b.p. 124–126° at 0.15 mm. The infrared spectrum was identical with that of III prepared as described below.

Anal. Calcd. for $C_9H_{12}N_2O_2$: C, 59.99; H, 6.71; N, 15.55. Found: C, 59.96; H, 6.73; N, 15.45.

Ethyl N-(3-Pyridylmethyl)carbamate (III).—With minor changes, the method used for synthesis of the 2-pyridyl isomer[17] was followed. 3-Picolylamine (10.0 g., 0.093 mole, Reilly Tar and Chemical Corp.) and triethylamine (9.35 g., 0.093 mole) were dissolved in 100 ml. of dry ether and a solution of ethyl chloroformate (10.0 g., 0.092 mole) in 50 ml. of dry ether was added dropwise during 1.5 hr. The mixture was stirred for a further 30 min. and then filtered. The filtrate was distilled to give III (8.27 g., 49%), b.p. 120° at 0.14 mm.

Hydrolysis of the N-Ethoxycarbonyl Compounds II.—Each of the bases IV was prepared by heating the corresponding ethoxycarbonyl compound II in a stainless steel autoclave with a 10% solution of potassium hydroxide in water–ethanol (1:1). The best yield in each case was obtained when the volume of solution per gram of starting material, temperature, and duration of heating were for IIa, 30 ml., 125°, 3.5 hr.; for IIb, 60 ml., 110°, 4.5 hr.; and for IIc, 30 ml., 150°, 4 hr. After hydrolysis, the basic solution was distilled until most of the ethanol had been evolved and was then extracted with ether. The ether solution was washed with a little 10% aqueous sodium carbonate and dried with magnesium sulfate. Evaporation of the solvent gave a brown oil, which was distilled under reduced pressure.

The bases (IV) were colorless or pale yellow oils which darkened on exposure to air. It was not possible to obtain a close elemental analysis for IVb which was particularly unstable; however, this compound gave a chloroplatinate which was analytically pure without recrystallization. The dipicrates were prepared in aqueous solution and recrystallized from 0.5% aqueous picric acid.

***dl*-Anatabine** (1,2,3,6-tetrahydro-2,3'-bipyridine, IVa) was produced in 56% yield, b.p. 136° at 6.5 mm. (lit.[3a] b.p. 145–146° at 10 mm. for *l*-anatabine).

Anal. Calcd. for $C_{10}H_{12}N_2$: C, 74.97; H, 7.55; N, 17.48. Found: C, 75.05; H, 7.57; N, 17.23.

The **dipicrate** of IVa had m.p. 198–200° dec. (lit.[3b] m.p. 201–201.5°).

Anal. Calcd. for $C_{22}H_{18}N_8O_{14}$: C, 42.73; H, 2.93; N, 18.12. Found: C, 42.82; H, 3.23; N, 17.98.

4-Chloro-1,2,3,6-tetrahydro-2,3'-bipyridine (IVb) was produced in 35% yield, b.p. 125° at 0.4 mm.; its **dipicrate** had m.p. 223–225° dec.

Anal. Calcd. for $C_{22}H_{17}ClN_8O_{14}$: C, 40.47; H, 2.63; N, 17.16. Found: C, 40.79; H, 2.85; N, 16.90.

(15) H. Bowlus and J. A. Nieuwland, *J. Am. Chem. Soc.*, **53**, 3835 (1931).

(16) W. H. Carothers, I. Williams, A. M. Collins, and J. E. Kirby, *ibid.*, **53**, 4203 (1931).

(17) K. Winterfeld and H. Schüler, *Arch. Pharm.*, **293**, 203 (1960).

The **chloroplatinate** of IVb decomposed without melting.

Anal. Calcd. for $C_{10}H_{12}Cl_7N_2Pt$: C, 19.87; H, 2.17; Pt, 32.27. Found: C, 19.94; H, 2.22; Pt, 32.30.

1,2,3,6-Tetrahydro-4,5-dimethyl-2,3′-bipyridine (IVc) was produced in 76% yield, b.p. 108–111° at 0.25 mm.

Anal. Calcd. for $C_{12}H_{16}N_2$: C, 76.56; H, 8.57; N, 14.88. Found: C, 76.40; H, 8.63; N, 15.14.

The **dipicrate** of IVc had m.p. 170–172°.

Anal. Calcd. for $C_{24}H_{22}N_8O_{14}$: C, 44.59; H, 3.43; N, 17.33. Found: C, 44.55; H, 3.61; N, 17.39.

Lithium Aluminum Hydride Reduction of IIa and IIc.—A solution of the N-ethoxycarbonyl compound IIa (0.51 g., 0.0022 mole) in 5 ml. of dry monoglyme was added to a refluxing suspension of lithium aluminum hydride (0.50 g. 0.0132 mole) in 15 ml. of monoglyme during a 2-hr. period. The mixture was then refluxed for 8 hr. and allowed to cool. Excess reducing agent was decomposed with water–monoglyme solution (1:10) and the mixture was filtered. The filter cake was washed with ether, and the filtrate and washings were concentrated to a yellow oil (0.40 g.). This was distilled at 150° (air bath) under a pressure of 6.5 mm. to give 0.107 g. (28%) of **1,2,3,6-tetrahydro-1-methyl-2,3′-bipyridine** (*dl*-N-methylanatabine, Va). This material showed only one peak on gas chromatography; the infrared spectrum showed the N-methyl group (2795 cm.$^{-1}$) and that carbonyl and –NH– groups were absent. The n.m.r. spectrum confirmed the presence of two olefinic protons (δ = 5.63 p.p.m.). and of the N-methyl group (singlet, 2.00 p.p.m.), and showed the pattern characteristic of the 3-pyridyl group. The **dipicrate** prepared from Va in aqueous solution was recrystallized from 0.5% aqueous picric acid (decomposed above 200°, m.p. 222–224°, estimated by introduction of samples into preheated baths; lit.[3c] m.p. 207–208° for *l*-N-methylanatabine).

Anal. Calcd. for $C_{23}H_{20}N_8O_{14}$: C, 43.68; H, 3.19; N, 17.72. Found: C, 44.01; H, 3.45; N, 17.60.

When treated similarly the N-ethoxycarbonyl compound IIc gave **1,2,3,6-tetrahydro-1,4,5-trimethyl-2,3′-bipyridine** (Vb), b.p. 92° at 0.2 mm. (31%); **dipicrate**, m.p. 183–185°.

Anal. Calcd. for $C_{25}H_{24}N_8O_{14}$: C, 45.46; H, 3.66; N, 16.96. Found: C, 45.15; H, 3.88; N, 16.95.

Dehydrogenation of *dl*-Anatabine[3b,4] (IVa).—Synthetic *dl*-anatabine (0.200 g.) was heated with 10% palladium on charcoal (0.030 g.) at 200° for 20 min. in an atmosphere of nitrogen. The mixture was taken up in ether, the solution was filtered, and the solvent was distilled to leave a residue recognizable by its infrared spectrum[15] as 2,3′-bipyridine. This material gave a precipitate with saturated aqueous picric acid which was recrystallized three times from 0.5% aqueous picric acid,[15] m.p. and m.m.p. 161–163° with an authentic sample of 2,3′-bipyridine (lit.[15] m.p. 166–167°).

Comparison of IVa with Natural *l*-Anatabine.—A sample of natural *l*-anatabine was obtained by decomposition of the dipicrate. The natural and synthetic alkaloids were indistinguishable by gas chromatography on two columns. Retention volumes are given relative to nicotine which was used as an internal standard: column 1 at 198°, relative retention volume = 2.15; column 2 at 197°, relative retention volume = 2.85. The alkaloids also showed the same behavior in two paper chromatographic systems: (1) *t*-amyl alcohol–acetate buffer,[4] R_f 0.36; (2) *n*-butyl alcohol–pyridine–water,[8] R_f = 0.22.

Acknowledgment.—We are indebted to the American Tobacco Company for financial support of this work, and for providing a sample of natural *l*-anatabine dipicrate. P. M. Q. also thanks the Committee on the International Exchange of Persons for a Fulbright Travel Scholarship.

Configuration of N,β-Dimethylleucine, a Constituent Amino Acid of Triostin C

JUN-ICHI SHŌJI, KAZUO TORI, AND HIDEO ŌTSUKA

Shionogi Research Laboratory, Shionogi and Company, Ltd., Fukushima-ku, Osaka, Japan

Received December 8, 1964

The configurations of N,β-dimethylleucine derived from triostin C and two synthesized diastereoisomers (Ib and IIb), separated by ion-exchange chromatography, were studied by n.m.r. spectroscopy. The coupling constants obtained from the doublet signal due to the proton on the α-carbon atom of these three compounds were compared with those of the reference compounds, *i.e.*, N-methyl-L-isoleucine, N-methyl-DL-isoleucine (Ia), and N-methyl-DL-alloisoleucine (IIa). The information obtained from the n.m.r. spectra and other facts led to the conclusion that the configurations of N,β-dimethylleucine isomers Ib and IIb and the one derived from triostin C are represented by Fischer projection formulas I (DL), II (DL), and II (L) (R = CH_3), respectively. (These can be called N,γ-dimethyl-DL-isoleucine, N,γ-dimethyl-DL-alloisoleucine, and N,γ-dimethyl-L-alloisoleucine, respectively.)

N,β-Dimethylleucine was first discovered in nature by Sheehan and co-workers[1] from the degradation of ethamycin. They proved that the amino acid belongs to the L-series and that the configuration at the β-carbon atom is identical to that of the ergosterol side chain. As the absolute configuration at C-24 of the ergosterol side chain has been determined to be 24β,[2] the configuration of the N,β-dimethylleucine isolated from etamycin could be deduced. Later, Sheehan and Howell[3] synthesized and resolved β-methylleucine and related compounds to find an approach to clarify the whole configuration of N,β-dimethylleucine.

We also had isolated N,β-dimethylleucine from the degradation product of the antibiotic triostin C.[4] In order to elucidate the configuration at the β-carbon atom of the amino acid, we compared diastereoisomers of N,β-dimethylleucine with similar diastereoisomeric compounds such as N-methylisoleucine and N-methylalloisoleucine by n.m.r. spectroscopy, which was expected to reflect the relative configuration at the α- and β-carbon atoms of the diastereoisomers. Some other properties such as solubility and behavior on chromatography were also used for the comparison. N,β-Dimethylleucine was synthesized and separated into two diastereoisomers (Ib and IIb) by ion-exchange chromatography. A synthetic N-methylisoleucine-N-methylalloisoleucine mixture was also separated in a similar way. The coupling constants, $J_{H\alpha,H\beta}$, of these compounds were measured from the doublet signal of the proton on the α-carbon atom. The configurations of the N,β-dimethylleucine isomers were determined by reference to the relationships between the configurations and the coupling constants of the known compounds.

(1) J. C. Sheehan, H. G. Zachau, and W. B. Lawson, *J. Am. Chem. Soc.*, **80**, 3349 (1958).

(2) (a) W.-Y. Huang and C.-W. Hsu, *Hua Hsueh Hsueh Pao*, **28**, 68 (1962); *Chem. Abstr.*, **59**, 14047 (1963); (b) G. D. Maio and A. Romeo, *Gazz. chim. ital.*, **89**, 1627 (1959).

(3) J. C. Sheehan and M. G. Howell, *J. Org. Chem.*, **28**, 2279 (1963).

(4) H. Ōtsuka and J. Shōji, *J. Antibiotics* (Tokyo), **A16**, 52 (1963).