CHEMISTRY OF NYBOMYCIN

II. SYNTHESIS OF 4,5-DIMETHYL-2,7-DIOXO-1,2,7,8-TETRAHYDRO-1,8-DIAZAANTHRACENE[1]

GRAHAM LEADBETTER AND KENNETH L. RINEHART, JR.
Noyes Chemical Laboratory, University of Illinois, Urbana, Illinois

Received November 13, 1964

Dedicated to Professor R. B. Sandin on the Occasion of his Sixty-Eighth Birthday

ABSTRACT

4,5-Dimethyl-2,7-dioxo-1,2,7,8-tetrahydro-1,8-diazaanthracene, a compound previously obtained from phosphorus – hydrogen iodide reduction of deoxynybomycin, has been synthesized in eight steps from *m*-xylene, in 5% overall yield.

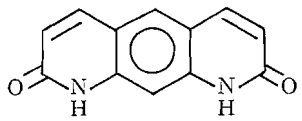
The water-insoluble antibiotic nybomycin (1, 2) was recently assigned (3) structure I (1-formyl-4-hydroxymethyl-5,8-dimethyl-2,7-dioxo-1,2,7,8-tetrahydro-1,8-diazaanthracene). In the assignment of structure I, a key degradation product was compound III (4,5-dimethyl-2,7-dioxo-1,2,7,8-tetrahydro-1,8-diazaanthracene), one of three products isolated from reduction of deoxynybomycin (II) with phosphorus – hydrogen iodide at 240° in a sealed tube.

Compound III was of particular significance in the structural studies on nybomycin, since it alone of the three reduction products (III, IV, and V) retained the basic ring system and fundamental chromophoric unit of nybomycin. Compound III was converted by treatment with dimethyl sulfate in base to its N,N′-dimethyl derivative (VI), which proved of prime importance in deduction of the ring system of nybomycin from nuclear magnetic resonance (n.m.r.) considerations. The present work reports the preparation of the two degradation compounds III and VI, both as confirmation of their structures and as an approach to the development of routes to more complex compounds containing the nybomycin ring system.[2]

In the present preparation *m*-xylene was treated with concentrated nitric and sulfuric acids at room temperature to give 4,6-dinitro-1,3-dimethylbenzene (VIII) (5) in 30% yield, together with the 2,4-dinitro isomer IX in 7% yield. Their previously assigned structures are confirmed by the n.m.r. coupling constants of the two isomers. In the spectrum of VIII, whose two aryl protons are para to one another, the singlets ($J < 1$ c.p.s.) expected (6) are found at τ 1.32 and τ 2.54 (Table I), while the ortho aryl protons of IX, at τ 1.76 and τ 2.36, have the expected (6) large coupling constant ($J = 9$ c.p.s.).

Oxidation of VIII to 4,6-dinitro-1,3-benzenedicarboxylic acid (X) was effected by chromium trioxide in concentrated sulfuric acid at approximately 15° (7). In addition to the dicarboxylic acid X, the monocarboxylic acid XI was always isolated. Chemical shift

[1]*For paper I in this series, see ref. 3.*
[2]*The unsubstituted ring system (VII) of compounds I, II, III, and VI had been prepared previously (4), but unfortunately before the widespread reporting of spectral data.*



VII

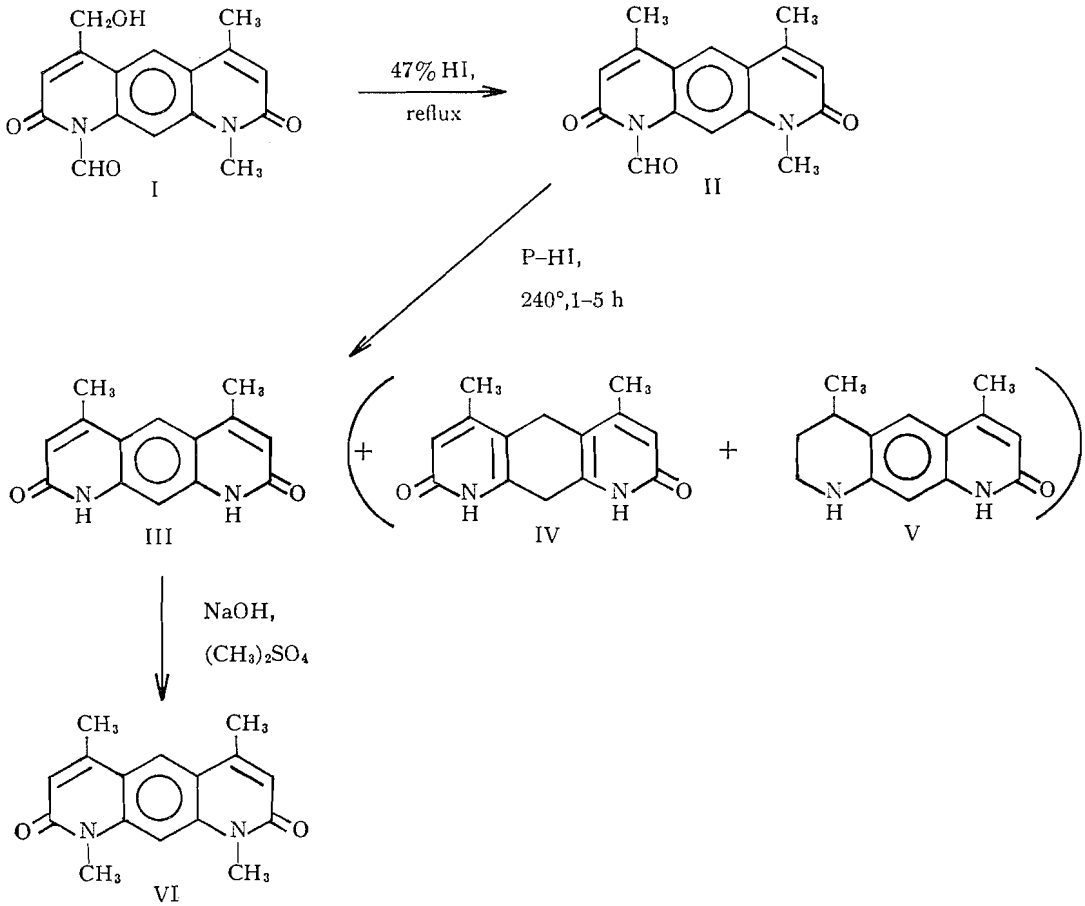
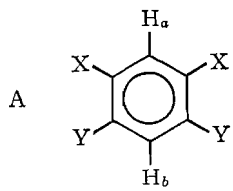


TABLE I

Chemical shift values (τ) for the protons of compounds having structure A*



Compound	H_a	H_b	CH_3—	$=C\overset{H}{<}$	Solvent
VIII (X = CH_3, Y = NO_2)	τ 2.54	τ 1.32	τ 7.30		$CDCl_3$
X (X = COOH, Y = NO_2)	1.80	1.34	—		D_2O
XIV (X = $COCH_3$, Y = NO_2)	2.46	1.10	7.40		CH_2Cl_2
	2.22	0.96	7.20		CF_3COOH
XVI (X = $COCH_3$, Y = NH_2)	1.00	2.80	7.10		CF_3COOH
XVII (X = $COCH_3$, Y = $NHCOCH_3$)	1.10	1.10	7.04, 7.50		CF_3COOH
III	1.80	1.16	7.08	2.76	CF_3COOH
VI	1.76	1.10	7.04, 5.70	2.60	CF_3COOH

*All peaks are singlets.

values for the dicarboxylic acid are reported in Table I; the monocarboxylic acid showed only singlets, at τ 1.30 (H-5), τ 2.12 (H-2), and τ 7.20 (aryl methyl).

Two routes were developed for conversion of the diacid X to 1,3-diacetyl-4,6-dinitrobenzene (XIV) via the known (7) diacid chloride XII. Conversion of XII to XIV by treatment of the former compound with diazomethane and subsequent reduction of the bis-diazoketone XIII with hydriodic acid gave XIV in yields as high as 59%. The yield of XIV by the alternative route, treatment of XII with diethyl magnesiomalonate, followed by concurrent hydrolysis and decarboxylation of the dimalonic ester XV, was approximately the same (53%). While the route via XV is somewhat longer and less convenient, it is preferred in view of the potential hazards of explosion and poisoning in working with large quantities of diazomethane and diazoketones.

The diacetyldinitrobenzene (XIV) was reduced by copper in sulfuric acid in 80% yield to 1,3-diacetyl-4,6-diaminobenzene (XVI)[3] and identified by melting point (7) and n.m.r. spectrum (Table I). Acetylation of XVI gave 94% of 1,3-diacetyl-4,6-diacetamidobenzene (XVII), characterized by its amide stretching absorption, elemental analyses, and n.m.r. spectrum (Table I).

Potassium *t*-butoxide catalyzed ring closure of XVII gave compound III in 69% yield. The overall yield of compound III from 4,6-dinitro-1,3-dimethylbenzene (VIII) was thus 15% by the diazomethane route and 14% by the magnesiomalonate route (5% and 4%, respectively, based on *m*-xylene).[4] Like nybomycin and related compounds, synthetic compound III did not melt below 350°. The infrared, ultraviolet, and n.m.r. (Table I) spectra of synthetic compound III matched those reported for the degradation product (8). As further proof of identity, synthetic compound III was converted by methylation to synthetic compound VI. For VI, too, n.m.r., ultraviolet, and infrared spectra (Fig. 1) were identical with those of the methylation product obtained in three steps from nybomycin (I → II → III → VI).

With completion of this synthesis, the heterocyclic skeleton of nybomycin is assured. Future efforts will be directed toward synthesis of derivatives of this ring system.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot stage and are uncorrected. Nuclear magnetic resonance spectra and microanalyses were obtained at the University of Illinois by D. H. Johnson, J. Nemeth, and their associates. Infrared spectra were obtained with a Perkin–Elmer double-beam recording spectrophotometer, model 237, on chloroform solutions or Nujol mulls except as noted in the text. Nuclear magnetic resonance spectra were obtained with a Varian high-resolution n.m.r. spectrometer, model A-60, in the solvents noted in the text. Ultraviolet spectra were obtained with a Beckman recording ultraviolet spectrophotometer, model DB, on ethanolic solutions.

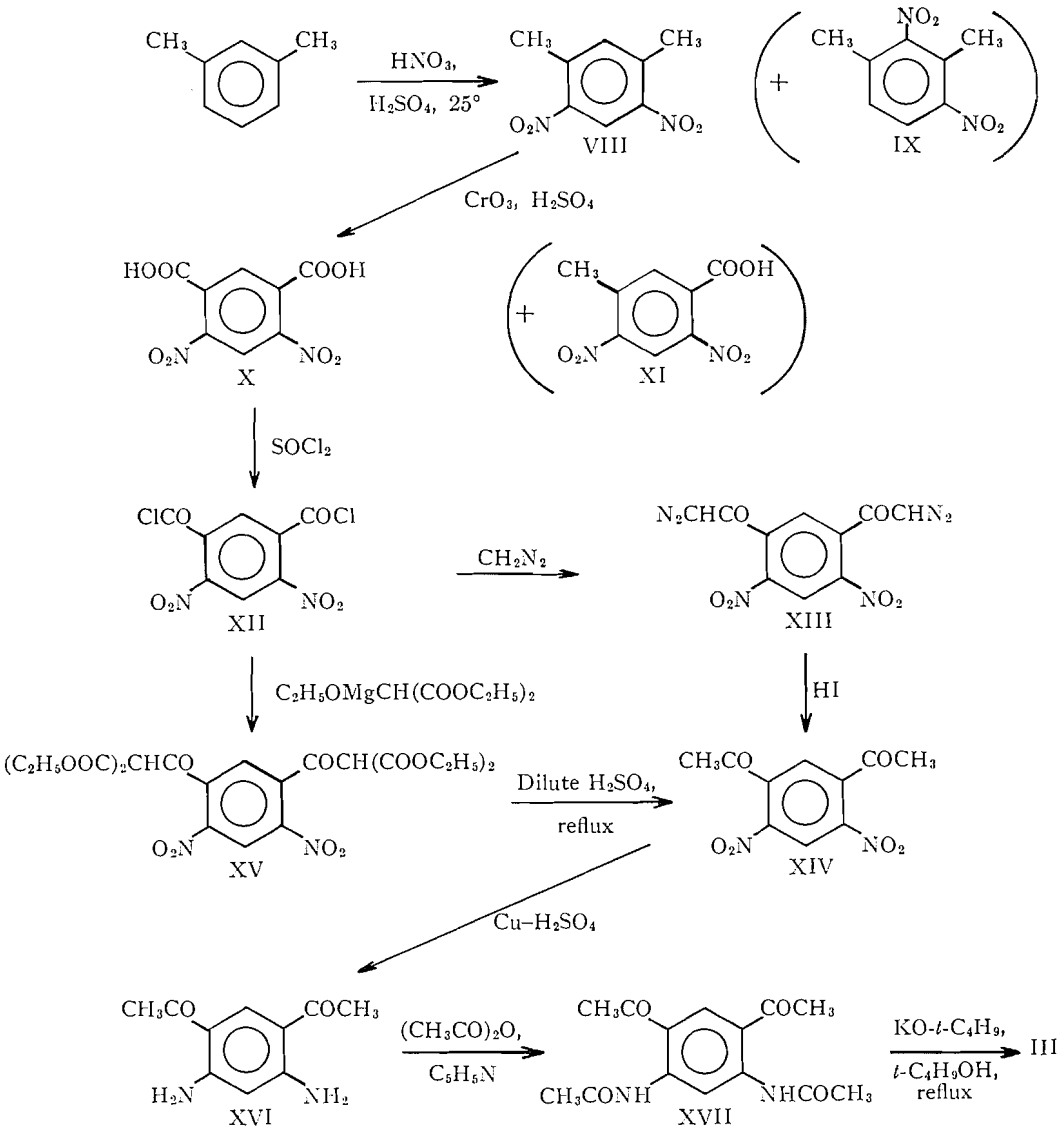
4,6-Dinitro-1,3-dimethylbenzene (VIII)

The procedure followed was that of Morgan (9). The crude product obtained from 200 ml of 70% nitric acid, 250 ml of 96% sulfuric acid, and 123 ml (ca. 106 g) of *m*-xylene weighed 150 g (76%). This was recrystallized repeatedly from 95% ethanol (5) until a constant melting product (59.5 g, 30%) was obtained. In five additional runs on a double scale the yields were 30–31%. 4,6-Dinitro-1,3-dimethylbenzene (VIII), m.p. 88–92° (lit. 89–93°) (5), was shown to be free of impurities by thin-layer chromatography over silica gel employing carbon tetrachloride solvent. The structure assigned was in accord with the compound's n.m.r. spectrum (Table I).

In addition to the desired 4,6-dinitro compound (VIII), 2,4-dinitro-1,3-dimethylbenzene (IX) could be isolated from the crude nitration product above by repeated recrystallization from chloroform (10). In

[3]*Ruggli and Reichwein (7) prepared XVI from XII via XIII, 4,6-dinitro-1,3-bischloracetylbenzene, and 4,6-diamino-1,3-bischloroacetylbenzene. Their yield of XVI from XII was approximately 44%, comparable to the present yields, but their route involved isolation of the intermediate bis-diazoketone (XIII) and one step more than either of the present routes.*

[4]*For comparison, the overall yield of compound VII from 4,6-dinitro-1,3-dimethylbenzene was approximately 10% (4).*



another run, from 135 g of mixed dinitroxylenes, the yield of crude 2,4-dinitro isomer (IX), shown to contain a small amount of the 4,6-dinitro isomer (VIII) by thin-layer chromatography, was 1.8 g (7%); the crude compound melted at 73–83° (lit. 82–83°) (10). Its n.m.r. spectrum (in deuterochloroform) contained a singlet at τ 7.54 (six protons, aryl methyl groups) and doublets (J = 9 c.p.s.) at τ 1.76 (one proton, H-5) and τ 2.36 (one proton, H-6).

4,6-Dinitro-1,3-benzenedicarboxylic Acid (X)

This compound was prepared exactly according to the oxidation procedure of Ruggli and Reichwein (7). From 20 g of 4,6-dinitro-1,3-dimethylbenzene (VIII), the yield of 4,6-dinitro-1,3-benzenedicarboxylic acid was 15.9 g (61%), m.p. 230–235° decomp. (lit. 234° decomp.) (7). Nuclear magnetic resonance data for the compound are presented in Table I. In 29 additional runs the yields varied from 29 to 51%.

The water-insoluble side product noted previously (7) was identified as a mixture of 4,6-dinitro-3-methylbenzoic acid (XI) and recovered starting material (VIII). A portion (1.2 g) of the material accumulated from several runs was separated into bicarbonate-soluble and -insoluble fractions. The base-soluble XI weighed 0.9 g and, after recrystallization from water, had m.p. 173–175° (lit. 171–171.5°) (11). Its infrared spectrum (Nujol) had a carbonyl band at 1 720 cm^{-1}; its n.m.r. spectrum (trifluoroacetic acid) contained only singlets, of area ratio 1:1:3 at τ 1.30 (H-5), τ 2.12 (H-2), and τ 7.20 (aryl methyl).

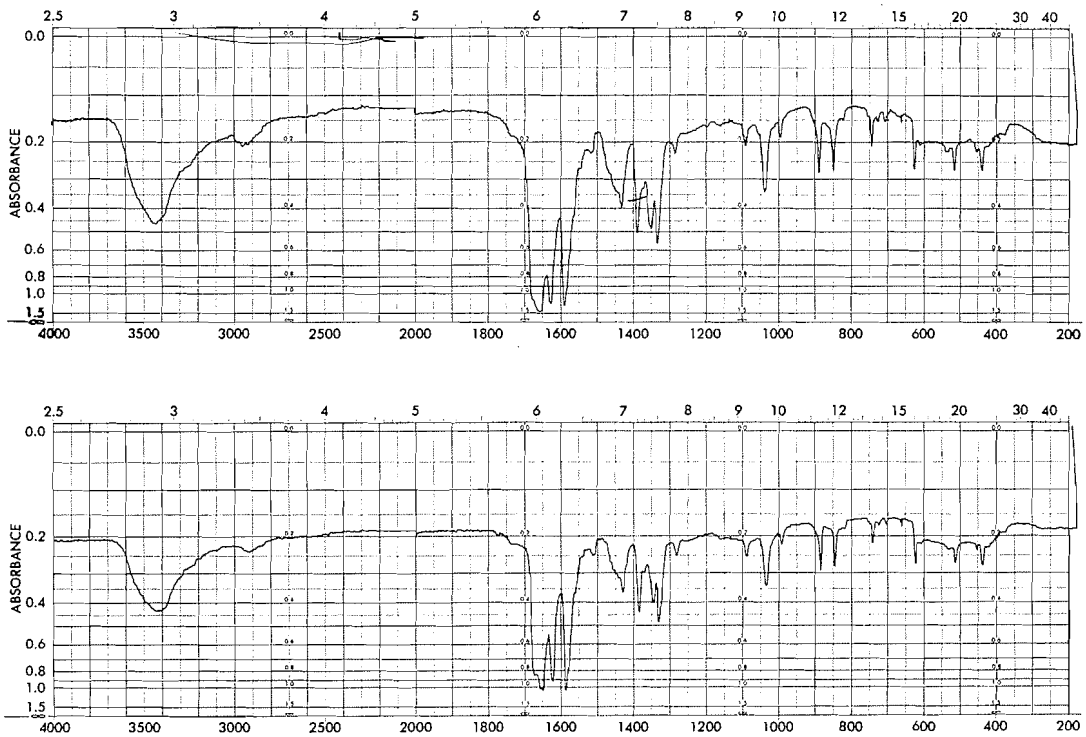


FIG. 1. Infrared spectra of 1,4,5,8-tetramethyl-2,7-dioxo-1,2,7,8-tetrahydro-1,8-diazaanthracene (VI), determined as potassium bromide pellets. Upper curve: sample obtained by methylation of 4,5-dimethyl-2,7-dioxo-1,2,7,8-tetrahydro-1,8-diazaanthracene (III) isolated from phosphorus – hydrogen iodide treatment of deoxynybomycin. Lower curve: sample obtained by methylation of synthetic compound III.

The bicarbonate-insoluble fraction weighed 95 mg and was identified as VIII, m.p. 86–88°.

Enhanced yields in the oxidation could be obtained by re-oxidizing the water-insoluble fraction. From each 20-g batch of material about 13 g of diacid was obtained. This would raise the yield of X by about 13% per run.

4,6-Dinitro-1,3-benzenedicarbonyl Chloride (XII)

The diacid chloride XII, m.p. 104–106° (lit. 106–108°) (7), was prepared in 82% yield by the exact procedure of Ruggli and Reichwein.

Preparation of 4,6-Dinitro-1,3-diacetylbenzene

A. Diethyl Malonate Procedure

The procedure followed was essentially that employed for the preparation of *o*-nitroacetophenone (12). From 27.1 g of magnesium turnings, 180.5 g of diethyl malonate, and 150 g of 4,6-dinitro-1,3-benzenedicarbonyl chloride (XII), the total yield of 4,6-dinitro-1,3-diacetylbenzene (XIV) was 71.2 g (53%). A single recrystallization from 95% ethanol gave the analytical sample, m.p. 137–138°.

Anal. Calcd. for $C_{10}H_8N_2O_6$: C, 47.62; H, 3.17; N, 11.11. Found: C, 47.31; H, 3.15; N, 11.12.

The infrared spectrum (chloroform) of the product contained a carbonyl band at 1 712 cm^{-1}. Its n.m.r. spectral data are found in Table I.

In a second run the yield was 52%.

B. Diazomethane Procedure

The procedure was essentially that of Wolfrom and Brown (11). A solution of 2.39 g (8.13 mmoles) of 4,6-dinitro-1,3-benzenedicarbonyl chloride (XII) in benzene was added dropwise to a stirred solution of 36.45 mmoles of diazomethane in ether (prepared from N-nitrosomethylurea and analyzed by decomposition with benzoic acid and titration with 0.2 *N* sodium hydroxide) (13). The yellow bis-diazoketone (XIII) precipitated and was filtered. It was then suspended in chloroform and stirred while 5 ml of 47% hydriodic acid was added; nitrogen was evolved and the mixture became deep purple. The chloroform solution was washed successively with water, sodium thiosulfate solution, sodium bicarbonate solution, and water; then

dried over magnesium sulfate; and finally evaporated to give 1.22 g (59%) of 4,6-dinitro-1,3-diacetylbenzene (XIV), m.p. 137–138° after two recrystallizations from 95% ethanol. The infrared spectrum and thin-layer chromatographic behavior (silica gel, chloroform solvent) of this sample were identical with those of the sample prepared by method A above. In four additional runs employing the diazomethane procedure, the yields of diacetyl compound (XIV) were 38–40%.

4,6-Diamino-1,3-diacetylbenzene (XVI)

From 948 mg of 4,6-dinitro-1,3-diacetylbenzene (XIV), the procedure of Ruggli and Reichwein gave 639 mg (80%) of 4,6-diamino-1,3-diacetylbenzene (XVI), m.p. 234–235° decomp. (lit. 235° decomp.) (7). An infrared spectrum (Nujol) of the product showed the expected two N—H stretching bands, at 3 500 and 3 320 cm^{-1}. Its n.m.r. values are found in Table I.

In a run on a 10-fold scale the yield was also 80%.

4,6-Diacetamido-1,3-diacetylbenzene (XVII)

The preparation followed a standard procedure (14). From 1.10 g of 4,6-diamino-1,3-diacetylbenzene (XVI) was obtained 1.51 g (94%) of 4,6-diacetamido-1,3-diacetylbenzene (XVII). Two recrystallizations from dimethylformamide gave the analytical sample, which did not melt below 350°.

Anal. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.78; H, 6.01; N, 10.43.

The infrared spectrum (Nujol) of the product contained amide bands at 3 250 cm^{-1} (N—H stretch) and 1 685 cm^{-1} (C=O stretch). Its n.m.r. spectral data are found in Table I.

4,5-Dimethyl-2,7-dioxo-1,2,7,8-tetrahydro-1,8-diazaanthracene (III)

The procedure was adapted from that of Camps (15). A mixture of potassium *t*-butoxide solution (prepared from 167 mg of potassium and 25 ml of dry *t*-butyl alcohol) and 500 mg of 4,6-diacetamido-1,3-diacetylbenzene (XVII) was heated under nitrogen for 1 h at reflux. Solid matter was filtered; weight, 244 mg. This was heated on the steam bath with 25 ml of 10% sodium hydroxide solution and then filtered hot. The filtrate was acidified with hydrochloric acid, and the resulting precipitate was filtered, washed with water, and dried; weight, 120 mg. Evaporation of the original *t*-butyl alcohol solution yielded further solid material, which was worked up with sodium hydroxide and acid as before to give an additional 179 mg of product. The total yield of compound III was thus 299 mg (69%); it did not melt below 350°. Its infrared (Nujol), ultraviolet, and n.m.r. (trifluoroacetic acid solution, see Table I) spectral data were the same as those of the product obtained from phosphorus – hydrogen iodide treatment of deoxynybomycin (8).

1,4,5,8-Tetramethyl-2,7-dioxo-1,2,7,8-tetrahydro-1,8-diazaanthracene (VI)

The dipyridone III (95 mg) dissolved in 25 ml of refluxing 10% sodium hydroxide solution. After addition of 2 ml of dimethyl sulfate a precipitate appeared; addition was continued until the solution was acidic to litmus. The precipitate was filtered, washed with water and acetone, and dried to give 62 mg (59%) of product (VI).

Anal. Calcd. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.67; H, 6.13; N, 10.24.

The compound did not melt below 350°, but its infrared (Nujol or potassium bromide, see Fig. 1), ultraviolet, and n.m.r. (trifluoroacetic acid, see Table I) spectra were all identical with those of the material obtained (8) from methylation of the phosphorus – hydrogen iodide product of deoxynybomycin.

ACKNOWLEDGMENT

This investigation was supported by Public Health Research grant No. AI-01278 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. F. Strelitz, H. Flon, and I. N. Asheshov. Proc. Natl. Acad. Sci. U.S. **41**, 620 (1955).
2. T. E. Eble, G. A. Boyack, C. M. Large, and W. H. Devries. Antibiot. Chemotherapy, **8**, 627 (1958).
3. K. L. Rinehart, Jr. and H. B. Renfroe. J. Am. Chem. Soc. **83**, 3729 (1961).
4. P. Ruggli, P. Hindermann, and H. Frey. Helv. Chim. Acta, **21**, 1066 (1938).
5. P. Ruggli, A. Zimmerman, and R. Thouvay. Helv. Chim. Acta, **14**, 1250 (1931).
6. L. M. Jackman. Applications of nuclear magnetic resonance spectroscopy in organic chemistry. Pergamon Press, New York. 1959.
7. P. Ruggli and H. Reichwein. Helv. Chim. Acta, **20**, 905 (1937).
8. H. B. Renfroe. Ph.D. Thesis, University of Illinois, Urbana, Illinois. 1961.
9. G. T. Morgan. J. Chem. Soc. **81**, 86 (1902).
10. R. Adams and A. S. Nagarkatti. J. Am. Chem. Soc. **72**, 1831 (1950).
11. M. L. Wolfrom and R. L. Brown. J. Am. Chem. Soc. **65**, 1516 (1943).
12. G. A. Reynolds and C. R. Hauser. Organic syntheses. Collective Vol. IV. John Wiley and Sons, New York. 1963. p. 708.
13. F. Arndt. Organic syntheses. Collective Vol. II. John Wiley and Sons, New York. 1943. p. 165.
14. N. D. Cheronis and J. B. Entrikin. Semimicro qualitative organic analysis; the systematic identification of organic compounds. 2nd ed. Interscience Publishers, Inc., New York. 1958. p. 410.
15. R. Camps. Arch. Pharm. **237**, 659 (1899).