remove thiophenol and then extracted with Claisen's alkali (three 100-ml portions). The latter extracts were diluted with water, neutralized with carbon dioxide, and then extracted with hexane. The combined hexane extracts were dried and concentrated to give 2.68 g (91%) of a 43:57 mixture of $\Delta^{9(11)}$-THC and Δ^{8}-THC.

An analytically pure sample was obtained by elution from silver nitrate–silica gel: nmr τ ($CDCl_3$) 9.12 (t, J = 7 Hz, $CH_3(CH_2)_4$), 8.95, 8.82 (s, $C(CH_3)_2$), 7.56 (t, J = 7 Hz, Ar CH_2), 6.26 (d, J = 12 Hz, Ar CH), 5.22 (s, 2 H, $C{=}CH_2$), 3.92, 3.83 (s, 2 H, Ar H); ir $\nu_{max}^{CCl_4}$ 3600 (OH), 890 cm^{-1} ($C{=}CH_2$); $[\alpha]^{25}D$ −38.5° (*c* 1.03, 95% EtOH).

Anal. Calcd *m/e* for $C_{21}H_{30}O_2$: 314.225. Found: 314.225.

11-Nor-9-ketohexahydrocannabinol 1-Methyl Ether.—$\Delta^{9(11)}$-THC 1-methyl ether (1.48 g, 4.51 mmol) in *tert*-butyl alcohol (742 ml) was treated with potassium carbonate (1.88 g) in water (100 ml), potassium permanganate (0.233, g, 1.48 mmol) in water (100 ml), and sodium metaperiodate (7.74 g, 35.4 mmol) in water (150 ml). After stirring at room temperature for 75 min, the mixture was extracted with benzene (three 500-ml portions) and the combined organic extracts were washed with saturated aqueous sodium bicarbonate (250 ml) and water (250 ml). After drying and concentrating, there remained 1.36 g of the desired product as a pale yellow oil of 99% purity. This product crystallized with difficulty from hexane: mp 87–88.2° (capillary); ir $\nu_{max}^{CCl_4}$ 1715 cm^{-1}; nmr τ ($CDCl_3$) 9.12 (t, J = 7 Hz, $CH_3(CH_2)_4$), 8.92, 8.78 (s, $C(CH_3)_2$), 7.48 (t, J = 7 Hz, Ar CH_2), 6.24 (s, 3 H, OCH_3), 3.78, 3.69 (s, 2 H, Ar H).

Anal. Calcd *m/e* for $C_{21}H_{30}O_3$: 330.219. Found: 330.220.

Registry No.—*trans*-$\Delta^{9(11)}$-THC, 27179-28-8; *trans*-$\Delta^{9(11)}$-THC 1-methyl ether, 27179-29-9; 11-nor-9-ketohexahydrocannabinol 1-methyl ether, 27179-30-2.

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Synthesis of 7-Dimethylamino-6-demethyl-6-deoxytetracycline (Minocycline) *via* 9-Nitro-6-demethyl-6-deoxytetracycline

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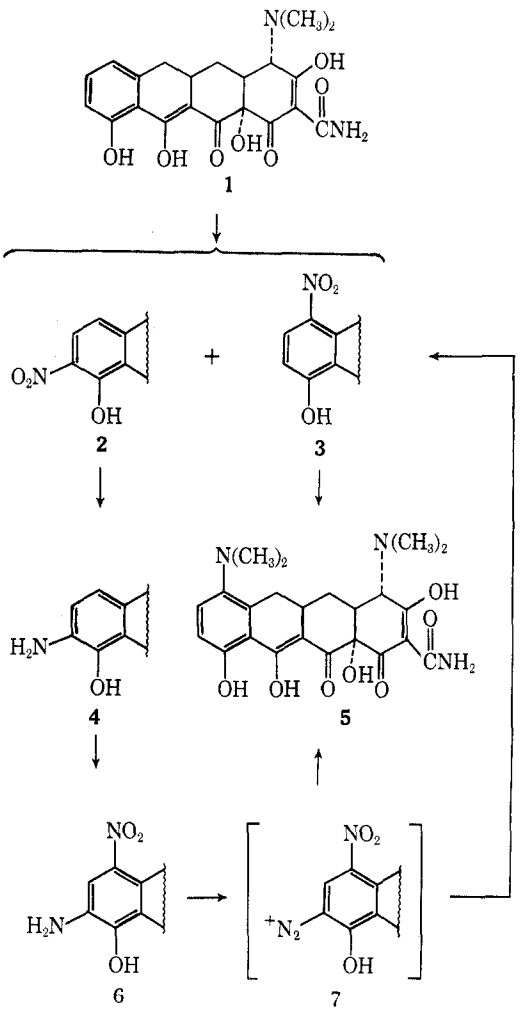
Recently it has been established that minocycline (7-dimethylamino-6-demethyl-6-deoxytetracycline, **5**) is a unique tetracycline derivative in that it is effective against tetracycline-resistant *staphylococci* in mice.[1,2] This important compound was originally prepared[1] by reductive methylation of 7-nitro-6-demethyl-6-deoxytetracycline (**3**) obtained by nitration of the accessible[3] 6-demethyl-6-deoxytetracycline (**1**). Unfortunately, the nitration of **1** affords a preponderance of the undesired 9-nitro isomer **2**, from which the 7-nitro isomer **3** must be separated.[4] Obviously, the efficiency of this process would be enhanced by the utilization of the 9-nitro isomer **2**.

The conversion of **2** to **5** has now been achieved *via* the previously reported[5] 9-amino-7-nitro intermediate **6**, obtained by catalytic reduction of **2** to the 9-amino derivative **4** followed by nitration.[6] The key feature of this sequence is the transformation of **6** to **5** by deamination at the 9 position *via* diazotization (butyl nitrite, sulfuric acid)[7] followed by reductive cleavage. Heating of the isolated diazonium salt **7** in ethanol affords the



7-nitro intermediate **3**,[8] convertible to the product **5** as previously described. This transformation, as well as reductive methylation, was accomplished in one operation by submitting **7**, prepared *in situ*, to hydrogenation with palladium catalyst in the presence of formaldehyde.[9]

(1) M. J. Martell and J. H. Boothe, *J. Med. Chem.*, **10**, 44 (1967).

(2) G. S. Redin, *Antimicrob. Ag. Chemother.*, 371 (1966); J. Federko, S. Katz, and H. Allnoch, *Amer. J. Med. Sci.*, **255**, 252 (1968); N. H. Steigbigel, C. W. Reed, and M. Finland, *ibid.*, **255**, 179, 296 (1968).

(3) J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk, *J. Amer. Chem. Soc.*, **82**, 3381 (1960).

(4) J. Petisi, J. L. Spencer, J. J. Hlavka, and J. H. Boothe, *J. Med. Chem.*, **5**, 538 (1962).

(5) J. L. Spencer, J. J. Hlavka, J. Petisi, H. M. Krazinski, and J. H. Boothe, *ibid.*, **6**, 405 (1963).

(6) A study of this reaction confirmed the reported conditions[5] as optimal. It may be noted that a twofold excess of nitrating agent resulted in much lower yields. Furthermore, nitration in liquid hydrogen fluoride gave apparently poorer results.

(7) J. J. Hlavka, A. Schneller, H. Krazinski, and J. H. Boothe, *J. Amer. Chem. Soc.*, **84**, 1426 (1962).

(8) For an earlier example of this reaction in the tetracycline series, see ref 7.

(9) The replacement of the diazonium group by hydrogen by this procedure is due either to hydrogenolysis or to reduction by formaldehyde. In either case it is unusual. Reduction of diazonium salts by formaldehyde is normally carried out in alkaline medium: R. Q. Brewster and J. A. Poje, *J. Amer. Chem. Soc.*, **61**, 2418 (1939). Hydrogenolysis of aromatic diazonium groups in the presence of palladium catalyst, a reaction first noted with another tetracycline by Dr. J. J. Hlavka of these laboratories, has no literature precedence of which we are aware.

The overall yield of the 7-dimethylamino product **5** from the 9-nitro isomer is 34%, and the yield obtainable directly from the 7-nitro isomer is 54%. Thus the efficiency of the nitration process for the preparation of **5** is dependent upon two factors: (1) the ratio of the nitro isomers, and (2) their total yield. We have been unable to improve the 7- to 9-nitro isomer ratio (about 1:2) although the nitration of **1** has been studied rather extensively. However, we have been able, by nitrating in liquid hydrogen fluoride,[10] to increase the total yield of separated nitro isomers to almost quantitative from the less than 50% observed when nitration is carried out in concentrated sulfuric acid. This procedure affords an 11.7% average yield (9.5–14.9%) of the 7-nitro isomer and 77.4% (73.5–80.6%) of the 9-nitro isomer, both of high purity.[11] The lower 7 to 9 isomer ratio is more than offset by the much higher total yield. Duplicate conversions of 6-demethyl-6-deoxytetracycline (**1**) to **5** using this procedure gave an average overall yield of 6.3% *via* the 7-nitro isomer and 26.4% *via* the 9-nitro isomer for a respectable total yield of 32.7%.

Experimental Section

All hydrogenations were carried out on a Parr hydrogenation apparatus at pressures of 5–35 psig. Solutions were dried over sodium sulfate and evaporations were carried out at reduced pressure. See ref 11 for bioassay data.

Nitration of 6-Demethyl-6-deoxytetracycline (1) in Liquid Hydrogen Fluoride.—A solution of 20.7 g (50 mmol) of 6-demethyl-6-deoxytetracycline (**1**) in 170 ml of liquid hydrogen fluoride was cooled in Dry Ice–acetone and treated with 5.06 g (50 mmol) of potassium nitrate. Nitrogen was blown over the solution while warming in a water bath for 30 min, and the black residue was evacuated (water aspirator) until no further gas evolution was noted. Cold acetone (80 ml) was added and the mixture was shaken to effect solution of the residue. After filtering and washing the acetone-insoluble material (KF·HF, 3.09 g, 78% of theoretical), the filtrate was poured into 2 l. of stirred ether. The mixture was stirred 35 min and filtered. The filtrate immediately developed additional precipitate; it was filtered again and combined with the previously obtained solid, and the entire yellow mass was washed with *ca.* 100 ml of ether. The crude nitration product weighed 26 g.

Separation of the Crude Mixed Nitro Isomers.—This method is a modification of the documented procedure.[4] The crude nitro product was suspended in 160 ml of methanol, and triethylamine was added with stirring to adjust the pH to 7.5. The mixture was stirred 45 min, maintaining (with triethylamine or sulfuric acid) the pH at 7.5 ± 0.2, and then filtered. The filter cake was washed with *ca.* 50 ml of methanol (the combined filtrates were saved for recovery of the 7-nitro isomer) and resuspended in 120 ml of methanol. The pH was adjusted to 6.5 with sulfuric acid, and the mixture was stirred 3 hr and filtered. The filter cake was washed with a small amount of methanol and dried *in vacuo* to constant weight to afford 18.21 g (79.0%) of a yellow solid (bioassay[11] 421).

The filtrate, after removal of the 9-nitro isomer **2**, was immediately treated with sulfuric acid to pH 1.0 and stirred at room temperature for 3 hr. The 7-nitro isomer **3** crystallized as the sulfate salt; it was filtered and washed with a few milliliters of methanol, and then dried *in vacuo* to constant weight to afford 2.99 g (10.6%) of a pale yellow powder (bioassay[11] 4254).

The results of four such experiments are given in Table I.

TABLE I

Expt no.	9-NO_2 isomer 2 Yield, %	9-NO_2 isomer 2 Bioassay[11]	7-NO_2 isomer 3 Yield, %	7-NO_2 isomer 3 Bioassay[11]
1	79.0	421	10.6	4254
2	73.5	594	14.9	3587
3	80.6	774	9.5	4360
4	76.6	665	11.8	3868
	Av 77.4		Av 11.7	

Reductive Methylation of 7-Nitro-6-demethyl-6-deoxytetracycline (3) to Minocycline (5) Hydrochloride Dihydrate.—The procedure described by Martell and Boothe[1] was used. All of the 7-nitro-6-deoxytetracycline, obtained from the four experiments described above, was blended and divided into two portions which were treated separately. The yields of 7-dimethylamino-6-demethyl-6-deoxytetracycline (**5**) disulfate obtained from the two preparations were 89.4 and 89.8% (bioassay[11] 509 and 469, respectively).

The disulfate salt was converted to the monohydrochloride dihydrate[12] by the following procedure.[13] A solution of 5.67 g of 7-dimethylamino-6-demethyl-6-deoxytetracycline (**5**) disulfate in 68 ml of water containing 0.226 g of sodium sulfite was adjusted to pH 6.5 at 25° by dropwise addition of 5 *N* sodium hydroxide. The solution was extracted successively with 145, 115, 115, and 115 ml of chloroform. The combined extracts were washed with 6 ml of saturated sodium chloride solution, dried, and evaporated to dryness (25° bath) to afford 4.5 g of a glass. The material was suspended in 8 ml of 1 *N* hydrochloric acid and the pH was adjusted to 1.1–1.3 with 6 *N* hydrochloric acid. The resulting solution was stirred for about 5 min with activated charcoal and filtered through acid-washed Celite,[14] and the filter cake was washed with 3 ml of 5% sodium chloride solution. The mother liquor was stirred, the pH was adjusted to a constant 4.0 with 5 *N* sodium hydroxide, and the mixture was then stirred at 5° for several hours. The product was collected by filtration and washed with 5 ml of 5% sodium chloride and 1.5 ml of 0.001 *N* hydrochloric acid. Drying at room temperature under reduced pressure for 24 hr afforded the crude hydrochloride of **5**.

The product was recrystallized as follows. A solution of 1.52 g of the hydrochloride was dissolved in 3.2 ml of 1 *N* hydrochloric acid, and the resulting solution was treated with 16 mg of activated charcoal for 10 min, followed by filtration through acid-washed Celite.[14] The cake was washed with 0.4 ml of 5% aqueous sodium chloride. The combined filtrate and wash was adjusted to pH 4.0 with 5 *N* sodium hydroxide. The suspension was stirred at 0° for several hours and then aged in the refrigerator overnight. The solid was collected, washed with 1 ml of 0.001 *N* hydrochloric acid, and dried at 40° *in vacuo* for several hours to give 1.43 g (94%) of product.

By this procedure, conversion of the two samples of the disulfate salt of 7-dimethylamino-6-demethyl-6-deoxytetracycline (**5**) gave the corresponding monohydrochloride dihydrate in yields of 59.4 and 60.2% (bioassay[11] 893 and 892, respectively). This product was identical by spectral and chromatographic analysis with an authentic sample of minocycline monohydrochloride dihydrate.

Reduction of 9-Nitro-6-demethyl-6-deoxytetracycline (2) to 9-Amino-6-demethyl-6-deoxytetracycline (4).—The 9-nitro-6-demethyl-6-deoxytetracycline (**2**) obtained from the four experiments described above was blended, and the combined material was then divided into two portions and hydrogenated as described previously.[5] The products of the two experiments were purified by conversion of the isolated disulfate salt to the neutral form[5] followed by reconversion to the disulfate in overall yields of 82.8 and 84.7% (bioassay[11] 1096 and 985, respectively).

Nitration of 9-Amino-6-demethyl-6-deoxytetracycline (4).—The procedure previously described was used.[5] The yields, in duplicate experiments, of 9-amino-7-nitro-6-demethyl-6-deoxytetracycline (**6**) obtained from the above-described **4** were 98.0 and 99.0% (bioassay[11] 3000 and 2734, respectively).

Conversion of 9-Amino-7-nitro-6-demethyl-6-deoxytetracycline (6) to Minocycline (5) Hydrochloride Dihydrate.—To 120 ml of ice-cold methanol containing 0.54 ml of concentrated sulfuric

(10) A preliminary study of this reaction was carried out by P. Bitha of these laboratories.

(11) Bioassays were measured by the turbidimetric assay of E. Pelcak and A. G. Dornbush, *Ann. N. Y. Acad. Sci.*, **51**, 218 (1948), using *Staphylococcus aureus* as the test organism. Results are compared to neutral tetracycline standard (1000), except for the case of minocycline (**5**) which was compared to minocycline neutral (1000). Standard *in vitro* biological activities for the other compounds discussed in this paper are as follows: 9-nitro-6-demethyl-6-deoxytetracycline (**2**), 200; 7-nitro-6-demethyl-6-deoxytetracycline (**3**), 6000; 9-amino-6-demethyl-6-deoxytetracycline (**4**), 1400; 7-nitro-9-amino-6-demethyl-6-deoxytetracycline (**6**), 4000.

(12) This salt was first prepared by Dr. J. Krueger and Mr. W. Barringer (Belgian Patent 696,488) and is recorded in ref 1.

(13) We wish to thank Dr. M. Tobkes for this procedure.

(14) Celite is the trademark of the Johns Manville Co. for diatomaceous earth silica products.

acid was added with stirring 6 g of 9-amino-7-nitro-6-demethyl-6-deoxytetracycline (6). To the resulting solution was added 2.03 ml (2 mol equiv) of *n*-butyl nitrite, and stirring was continued for 1.75 hr during which time a red solid separated. Urea[15] (540 mg) was added and stirring was continued for 15 min. After the addition of 19 ml of 40% aqueous formaldehyde, the solution was added to a suspension of 1.5 g of 10% palladium-on-carbon catalyst in 7 ml of ethylene glycol monomethyl ether. After an apparent induction period of about 1 hr, hydrogen uptake could be observed and was complete in about 45 min. The filtered solution was poured into 2 l. of ether and aged in the refrigerator overnight. The supernatant liquid was decanted and the residual solid taken up in 125 ml of methanol and reprecipitated from 1400 ml of ether to give 5.4 g (92.0%) of crude minocycline disulfate.

A duplicate experiment afforded 91.0% of product **5** disulfate. These products were then converted by the procedure described above to once-recrystallized minocycline monohydrochloride dihydrate in yields of 43.8 and 46.4% (bioassay[11] 960 and 930, respectively), identical by spectral and chromatographic analysis with an authentic sample.

Registry No.—**2**, 27298-24-4; **5**, 27179-27-7.

Acknowledgments.—The authors are indebted to Mr. L. Brancone and staff for microanalyses, and to Mr. A. Dornbush and Dr. J. J. Corbett and their staffs for biological assays. We are grateful to Drs. J. J. Hlavka, M. J. Martell, and R. Winterbottom, and to Miss P. Bitha for useful conversations. We also wish to acknowledge that the 9-nitro route to minocycline was first suggested by Dr. R. G. Wilkinson.

(15) The presence of a large excess of butyl nitrite was found to inhibit the subsequent hydrogenation.

Structure of Murrayacine[1]

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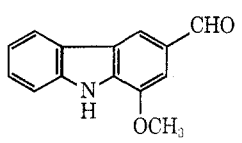
Since the report of the first carbazole alkaloid murrayanine[4] (I) from the stem bark of *Murraya koenigii*

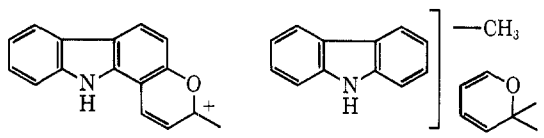


I

Spreng. (Family *Rutaceae*), study of carbazole alkaloids from the taxonomically related genera, *Murraya*, *Glycosmis*,[5,6] and *Clausena*[7] of the family *Rutaceae* has resulted in isolation of different carbazole alkaloids.[8,9] The present communication relates to the structure of one of these, murrayacine (II), which was isolated from the stem bark of *Murraya koenigii* Spreng. in poor yield.

Murrayacine (II), $C_{18}H_{15}NO_2$, mp 244–245° (M^+ 277), gave a 2,4-dinitrophenylhydrazone and reduced ammoniacal silver nitrate solution showing the presence of an aldehyde function. Its ir spectrum (KBr) showed peaks at 3250 (NH function), 1675 (carbonyl function), 1640, 1600 (unsaturation and aromatic group), and 895, 865, 740 cm^{-1} (substituted benzene derivative). Its uv spectrum [$\lambda_{max}^{ethanol}$ 226 mμ (log ϵ 4.60), 282 (4.57), 301 (4.58)] was very similar to those of 3-formylcarbazole,[10] murrayanine, and 1,4-dimethyl-3-formylcarbazole.[4] This suggested the presence of a 3-formylcarbazole chromophore in II.

The nmr spectrum of II (60 Mc in DMSO) showed signals at δ 10.68 (for an aldehyde function) and at δ 12.0 (for the NH function). One of the aromatic protons appeared as a singlet at δ 8.4 while the other four appeared as multiplets centered around δ 8.15 and 7.35. The sharp singlet for the six protons together with the doublets for one proton each at δ 7.00 and 5.95 (J = 10 cps/sec) revealed the presence of a 2,2-dimethyl-Δ^3-pyran ring. The high intensity mass spectral peak at m/e 262 (M − 15) was suggestive of the formation of the carbazolopyrilium ion (III) during mass spectral fragmentation. The mass spectrum also showed peaks at m/e 234 (M − 15 − 28) due to loss of mass 28 from III. All these data were consistent with the presence of a 3- or 6-formyl-2′,2′-dimethyl-$\Delta^{3'}$-pyranocarbazole skeleton. The isolation of carbazole by zinc dust distillation confirmed the carbazole skeleton in II.



III VI

An alcohol (IV), obtained by sodium borohydride reduction of II, had a uv spectrum strikingly similar to that of girinimbine (V), the first 2,2-dimethyl-Δ^3-pyranocarbazole from a plant source.[11] This suggested that an identical chromophore was present in the two compounds. Because murrayacine was obtained only in small quantity, information regarding the fusion of the pyran ring to the carbazole ring in II was based on the structure elucidation of girinimbine which was formulated by Chakraborty, *et al.*,[11] as VI.

We previously reported that ozonolysis of V furnished an α-hydroxyaldehyde (VII). Structure elucidation of this aldehyde would settle the structure of girinimbine. In the course of the present work, we isolated not only the aldehyde VII but also the corresponding α-hydroxy acid (VIII), in better yield. Zinc dust distillation of VIII furnished 3-methylcarbazole. This showed that the methyl group of V, VII, and VIII was attached to C-3 or C-6 of the carbazole nucleus.

(1) Part XXVI in the series Chemical Taxonomy. Part XXV: B. K. Chowdhury and D. P. Chakraborty, *J. Indian Chem. Soc.*, in press. A short communication on the subject appeared in *Chem. Commun.*, 967 (1968).

(2) Participated as a National Institute of Sciences (India) Research Fellow.

(3) Participated as a Junior Research Fellow in the C.S.I.R. (India) scheme entitled "Studies on Chemical Taxonomy in Relation to the Family *Rutaceae*."

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(5) D. P. Chakraborty, *Phytochemistry*, **8**, 769 (1969).

(6) D. P. Chakraborty and B. P. Das, *Sci. Cult. (Calcutta)*, **32**, 181 (1966).

(7) D. P. Chakraborty, K. C. Das, and A. Islam, *J. Indian Chem. Soc.*, in press.

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(10) G. Büchi and E. W. Warnhoff, *J. Amer. Chem. Soc.*, **81**, 4433 (1959). The uv data of the formyl carbazoles provided by Professor G. Büchi is gratefully acknowledged.

(11) D. P. Chakraborty, B. K. Barman, and P. K. Bose, *Sci. Cult. (Calcutta)*, **30**, 445 (1964).