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.

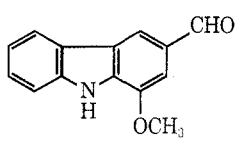
Structure of Murrayacine[1]

D. P. CHAKRABORTY,* K. C. DAS,[2] AND B. K. CHOWDHURY[3]

Bose Institute, Calcutta 9, India

Received June 18, 1970

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

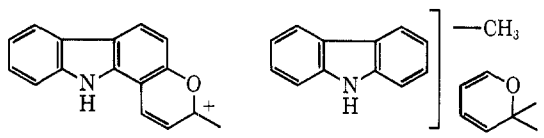


I

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*."

(4) D. P. Chakraborty, B. K. Barman, and P. K. Bose, *Tetrahedron*, **21**, 681 (1965).

(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.

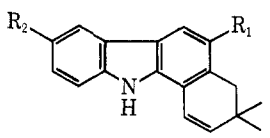
(8) B. K. Chowdhury and D. P. Chakraborty, *Chem. Ind. (London)*, 549 (1969).

(9) D. P. Chakraborty, A. Islam, S. P. Basak, and R. Das, *ibid.*, 593 (1970).

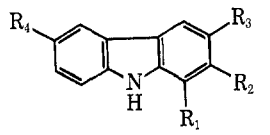
(10) G. Büchi and E. W. Warnhoff, *J. Amer. Chem. Soc.*, **81**, 4433 (1959). The uv data of the formyl carbazoles provided by Professor G. Büchi is gratefully acknowledged.

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

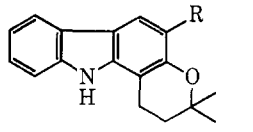
Furthermore, VII gave an *o*-acetate (IX), the uv spectrum of which [$\lambda_{max}^{ethanol}$ 226 mμ (log ε 4.56), 253 (4.09), 292 (4.22), 330 (3.74), and 372 (3.89)] was characteristic of a 1-formylcarbazole. Decarbonylation of VII resulted in a phenolic carbazole which could be methylated with diazomethane to the corresponding methoxycarbazole. The methoxy compound had a uv spectrum [$\lambda_{max}^{ethanol}$ 237 mμ (log ε 4.69), 258 (ε 4.36), 300 (4.15)] characteristic for 2-methoxycarbazole.[12] This showed that the phenol had the hydroxyl group at C-2 and could be formulated either as 2-hydroxy-3-methylcarbazole (X)[13] or 2-hydroxy-6-methylcarbazole (XI).[14] On comparison with synthetic specimens of X and XI, it has been found identical (mixture melting point, tlc, and uv) with X. On this basis, the hydroxyaldehyde could be formulated as VII and girinimbine as V.[15,16] The alcohol IV would, therefore, be IVa or IVb.



II, R_1 = CHO; R_2 = H
IVa, R_1 = CH_2OH; R_2 = H
IVb, R_1 = H; R_2 = CH_2OH
V, R_1 = CH_3; R_2 = H



VII, R_1 = CHO; R_2 = OH; R_3 = CH_3; R_4 = H
VIII, R_1 = CO_2H; R_2 = OH; R_3 = CH_3; R_4 = H
IX, R_1 = CHO; R_2 = $OCOCH_3$; R_3 = CH_3; R_4 = H
X, R_1 = R_4 = H; R_2 = OH; R_3 = CH_3
Xa, R_1 = R_4 = H; R_2 = OCH_3; R_3 = CH_3
XI, R_1 = R_3 = H; R_2 = OH; R_4 = CH_3



XII, R = CHO
XIII, R = CH_3

Dihydromurrayacine (XII) (M^+ 279; ν_{max}^{KBr} 3328, 1665, 1600, 872, 755 cm^{-1}) yielded dihydrogirinimbine (XIII) on $LiAlH_4$ reduction. Hence murrayacine was II, a structure which accounts for the deshielded one-proton singlet at δ 8.40 as being due to H-4. The alcohol IV was therefore IVa.

(12) D. P. Chakraborty, J. Dutta, and A. Ghosh, *Sci. Cult. (Calcutta)*, **31**, 529 (1965).

(13) D. P. Chakraborty, D. Chatterjee, and S. Ganguli, *Chem. Ind. (London)*, 1662 (1969).

(14) D. P. Chakraborty, D. Chatterjee, and B. K. Chowdhury, *J. Indian Chem. Soc.*, in press.

(15) A preliminary announcement of the structure of girinimbine (Proceedings of the IUPAC Symposium on the Chemistry of Natural Products, London, 1968, p 418) was made; the identity of the decarbonylated product, $C_{18}H_{11}NO$, had not yet been established by synthesis. On the basis of the spectral analogy between girinimbine and mahanimbine, Dutta and Quasim [*Indian J. Chem.*, **7**, 307 (1969)] advanced the same structure of girinimbine.

(16) (a) B. S. Joshi, *et al.*, came to the same conclusion in connection with some other work (Proceedings of the Indo-Soviet Symposium on the Chemistry of Natural Products, New Delhi, India, Feb 1970). Their approach and some experimental results are different. (b) The structure has also been confirmed by synthesis: A. Islam, Dr. of Philosophy Thesis, Calcutta University, Jan 1970; *J. Indian Chem. Soc.*, in press.

Experimental Section[17]

Isolation of Girinimbine and Murrayacine. Girinimbine (V).—Air-dried finely powdered stem bark of *Murraya koenigii* Spreng. was extracted with petroleum ether (bp 40–60°). The residue after removal of solvent, was taken up in dry benzene and chromatographed over alumina (500 g). Eluents were collected in fractions (150 ml each). The residue from the 16th to 21st fraction on washing with petroleum ether gave yellowish crystals (1 g), mp 171–172°. On further crystallizations from a mixture of benzene and petroleum ether (bp 40–60°) and subsequent sublimation, a substance melting at 176° was obtained. This was identical with girinimbine.[11]

Murrayacine (II).—On removal of eluents from fractions 76–85 (benzene–chloroform mixture), a yellowish brown semisolid residue was obtained which on crystallization from ethyl acetate furnished light yellow crystals (50 mg), mp 235–240°. This on sublimation [180° (0.05 mm)] and further crystallization from ethyl acetate melted at 244–245°.

Anal. Calcd for $C_{18}H_{15}NO_2$: C, 77.98; H, 5.41; N, 5.37. Found: C, 78.41; H, 5.67; N, 5.37.

2,4-Dinitrophenylhydrazone of II.—2,4-Dinitrophenylhydrazone was obtained in the usual way. The resulting product was washed with methanol. The residue melted at 280°.

Anal. Calcd for $C_{24}H_{19}N_5O_5$: N, 15.31. Found: N, 15.1.

Sodium Borohydride Reduction of Murrayacine to IV.—A solution of murrayacine (15 mg) in methanol (20 ml) was reacted with an excess of sodium borohydride at room temperature for 20 hr. Water and 10% hydrochloric acid were added to the mixture. It was extracted with ether, made acid free, and dried. On removal of the solvent, a compound, mp 200°, was obtained. It was recrystallized from methanol and was found homogeneous by paper chromatography: $\lambda_{max}^{ethanol}$ 238 mμ (log ε 4.56), 288 (4.16), and 330 (3.64).

Anal. Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.0. Found: C, 77.10; H, 5.93; N, 5.29.

Dihydromurrayacine (XII).—A mixture of murrayacine (30 mg), PtO_2 (50 mg), and absolute ethanol (20 ml) was stirred at room temperature for 3 hr in the presence of hydrogen. The mixture was filtered. On removal of the solvent, a solid product was obtained which on crystallization from methanol furnished a colorless compound, mp 176°.

Anal. Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.25; H, 5.83; N, 5.34.

Zinc Dust Distillation of Murrayacine. Isolation of Carbazole.—Murrayacine (100 mg) was thoroughly mixed with dry zinc dust (10 g) and was distilled[4] when a solid was obtained. The solid was dissolved in benzene and chromatographed over alumina (3 g). From the benzene eluent of the chromatogram, a compound, $C_{12}H_9N$, mp 225°, was obtained. This was identical with carbazole in all respects (mixture melting point, uv, and tlc).

Ozonolysis of Girinimbine.—Through the solution of V (1 g) in carbon tetrachloride (120 ml) was passed ozonized oxygen for 1.5 hr at −10° when no further uptake of ozone was noticed. Ice-cold water was added to the reaction mixture and heated on a water bath for 30 min to decompose the ozonide. The phenolic fraction isolated from the reaction mixture as a brown mass was dissolved in benzene. This was chromatographed over silica gel and 25 ml of each fraction was collected using benzene as an eluent. Fractions 5–9 yielded a compound, mp 186°, and fractions 9–16 gave a compound, mp 250°. The compound (50 mg) melting at 186° on recrystallization from benzene melted at 193°, identical with the hydroxyaldehyde VII previously obtained[11] (mixture melting point and uv). The compound VIII (100 mg) melting at 250° on recrystallization from benzene, melted at 255°: ν_{max}^{KBr} 3390, 1700 cm^{-1}.

Anal. Calcd for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.60; N, 5.81. Found: C, 69.43; H, 4.60; N, 5.89.

Acetylation of VII to IX.—The aldehyde VII (25 mg) was dissolved in pyridine and acetic anhydride (2 ml) and refluxed for

(17) Melting points were determined on a Koffler block. For chromatography, alumina by Sarabhai-Merck Co, and Merck silica gel were used. Ultraviolet spectra (95% ethanol as solvent) were recorded on Beckman DK-2 and Hilger and Watts uv spectrophotometers.

1 hr. The reaction mixture was poured into crushed ice and an almost colorless substance separated. On crystallization of the substance from benzene, IX, mp 217°, was obtained (20 mg): ν_{max}^{KBr} 3400, 1750, 1660 cm^{-1}.

Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.78; H, 4.82; N, 5.37.

Decarbonylation of VII.—The aldehyde VII (40 mg) was mixed with Pd/C (20 mg) and heated in a sealed tube with 1 ml of dry alcohol for 15 min at 270° under vacuum. The residue obtained, after removal of solvent from the alcoholic extract of the reaction product, on crystallization from benzene furnished crystals of X melting at 243°. The compound was soluble in 1% alkali and gave an olive green color with alcoholic ferric chloride solution. This was found identical with 2-hydroxy-3-methylcarbazole (mixture melting point, uv) and different from 2-hydroxy-6-methylcarbazole (mixture melting point 210–220°): $\lambda_{max}^{ethanol}$ 236 mμ (log ε 4.72), 258 (4.31), 302 (4.24).

Anal. Calcd for $C_{13}H_{11}NO$: C, 79.17; H, 5.62; N, 7.10. Found: C, 79.03; H, 5.57; N, 7.10.

Methylation of X.—The phenol X (40 mg) in methanol (15 ml), on treatment with diazomethane and keeping in a refrigerator for 16 hr, furnished a semisolid mass. This was dissolved in benzene and chromatographed over alumina (3 g). From the fractions collected with benzene as eluent, a colorless solid (Xa) was obtained which on crystallization from a mixture of benzene and petroleum ether melted at 225° (yield, 15 mg).

Anal. Calcd for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.54; H, 6.08; N, 6.81.

Reduction of Dihydromurrayacine to XIII.—A solution of XII (5 mg) in tetrahydrofuran (10 ml) was slowly added to a suspension of $LiAlH_4$ (1 gm) in tetrahydrofuran (7 ml). The mixture was refluxed for 3 hr. The $LiAlH_4$ was decomposed and the reaction mixture was extracted with ether. The ether layer was washed with water and dried, and solvent was removed from it. A solid, mp 176°, identical with dihydrogirinimbine (mixture melting point, tlc, uv) was obtained. No analysis was possible.

Zinc Dust Distillation of an α-Hydroxy Acid VIII. Formation of 3-Methylcarbazole.—The compound VIII (100 mg) was mixed with zinc dust (7.5 g) and distilled by the method and procedure described previously.[4] On working up the reaction product, a compound, mp 207°, was obtained. This was identified as 3-methylcarbazole[5] (mixture melting point, uv).

Registry No.—II, 27300-29-4; II 2,4-DNP, 27300-30-7; IVa, 27300-31-8; V, 23095-44-5; VIII, 27300-33-0, IX, 27300-34-1; X, 24224-30-4; Xa, 24224-28-0; XII, 17750-37-7.

Acknowledgment.—The authors thank Professor S. M. Sircar, Director, Dr. D. M. Bose, and Dr. A. Sen, Head of Department of Chemistry, for their interest in the work. The financial supports partly by C.S.I.R. and partly by NIS (India) are gratefully acknowledged, The authors acknowledge their indebtedness to Dr. B. C. Das of the Institute-de-Chimie des Substances, Gif-Sur-Yvette, France, for nmr and mass spectra.

The Preparation and Properties of Some Cytosine Derivatives

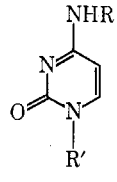
R. S. GOODY AND R. T. WALKER*

Chemistry Department, The University of Birmingham, Birmingham 15, England

Received July 6, 1970

In order to develop a method for the preparation of some O-acylated derivatives of cytosine, in particular, 2′,3′,5′-tri-*O*-benzoylcytidine (IV), a study was made of some 4-N-acylated cytosines in an attempt to obtain a derivative which could be deacylated at 4 N using conditions under which the sugar benzoyl groups would be stable. The only methods available for obtaining partially acylated derivatives of cytidine give the 4-*N*-acyl derivative as the final product[1] or partially O-acylated derivatives.[2] A preliminary communication of part of this work has already appeared,[3] and from this study it was apparent that in aqueous media, of the derivatives investigated (4-*N*-trifluoroacetyl-, trichloroacetyl-, dichloroacetyl-, monochloroacetyl-, and acetylcytosine), only the latter two would be of use as a protecting group for 4 N because of the lability of the other derivatives.

4-*N*-Chloroacetylcytosine (I) was prepared as previously described.[3] This was easily converted into cytosine under mild acidic conditions, the time for 50% hydrolysis in 0.1 *N* HCl at 20° being 13.5 min, compared with 130 min for the hydrolysis of 4-*N*-acetylcytosine (I). The compound was indefinitely stable in dry ethanol and dry pyridine. It has previously been reported[4] that the acidic deacylation of 4-*N*-acetylcytosine (80% acetic acid) gave a mixture of cytosine and uracil in equal amounts. In the present study, no chromatographic evidence was found for the presence of any uracil (<5%) in the acidic hydrolysate of either 4-*N*-chloroacetyl- or 4-*N*-acetylcytosine.

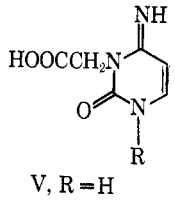


I, R^1 = H; R = $CH_2ClC(=O)-$

II, R^1 = 2′,3′,5′-tri-*o*-chloroacetylribose; R = $CH_2ClC(=O)-$

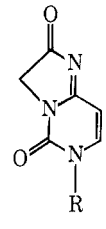
III, R^1 = 2′,3′,5′,-tri-*o*-benzoylribose; R = $CH_3C(=O)-$

IV, R^1 = 2′,3′,5′,-tri-*o*-benzoylribose; R = H



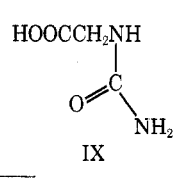
V, R = H

VI, R = ribose

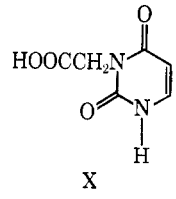


VII, R = H

VIII, R = 2′,3′,5′-tri-*o*-chloroacetylribose



IX



X

(1) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Amer. Chem. Soc.*, **85**, 3821 (1963); K. A. Watanabe and J. J. Fox, *Angew. Chem.*, **78**, 579 (1966); G. Keith and J. P. Ebel, *Biochim. Biophys. Acta*, **166**, 16 (1968).

(2) C. B. Reese and D. R. Trentham, *Tetrahedron Lett.*, 2459 (1965); F. Eckstein and F. Cramer, *Chem. Ber.*, **98**, 995 (1965).

(3) R. S. Goody and R. T. Walker, *Tetrahedron Lett.*, 289 (1967).

(4) D. M. Brown, A. R. Todd, and S. Vadarajan, *J. Chem. Soc.*, 2384 (1956).