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MUKOEIC ACID, THE FIRST CARBAZOLE CARBOXYLIC ACID FROM A PLANT SOURCE*

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Abstract—Mukoeic acid, isolated from the bark of *Murraya koenigii* had been identified as 1-methoxy-carbazole-3-carboxylic acid (II).

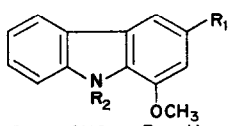
INTRODUCTION

SINCE the report of murrayanine[1,2] (I), from *Murraya koenigii* Spreng., we reported several carbazole alkaloids from genera, *Glycosmis*[3,4] *Murraya*[5] and *Clausena*,[6] of the same sub-family Aurantiae of the Rutaceae.

All these carbazoles have a C—CH_3 or C—CHO group at C-3. In view of the recent demonstration[7] that aniline can give rise to ^{14}C-methyl-*p*-toluidine and then by oxidation to *p*-hydroxymethylaniline, the aromatic formyl group in the carbazoles of the Rutaceae could be considered as oxidative variants of the aromatic C-methyl group. Therefore, we were interested to see if carbazoles with hydroxymethyl or carboxylic acid groups occurred in *Murraya koenigii* Spreng. In the present communication, we report the structural studies of mukoeic acid (II), the first carbazole carboxylic acid from a plant source.

RESULTS

From the alcoholic extract of the stem bark of *Murraya koenigii* Spreng., we isolated a homogeneous (TLC), optically inactive acidic compound, $C_{14}H_{11}NO_3$, m.p. 242°. It had



I. R_1 = —CHO ; R_2 = H
II. R_1 = —COOH ; R_2 = H
III. R_1 = COOMe ; R_2 = CH_3
IV. R_1 = COOMe ; R_2 = H
V. R_1 = R_2 = H

* Part XXVIII in the series 'Chemical Taxonomy'; for Part XXVII, see D. P. CHAKRABORTY and A. ISLAM, *J. Indian Chem. Soc.* **48**, 91 (1971). Short communication on the subject appeared in *Chem. & Ind*, 549 (1969).

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[5] D. P. CHAKRABORTY, A. ISLAM, S. P. BASAK and R. DAS, *Chem. & Ind.* 593 (1970) and previous references.
[6] D. P. CHAKRABORTY, K. C. DAS and A. ISLAM, *J. Indian Chem. Soc.* **47**, 1197 (1970).
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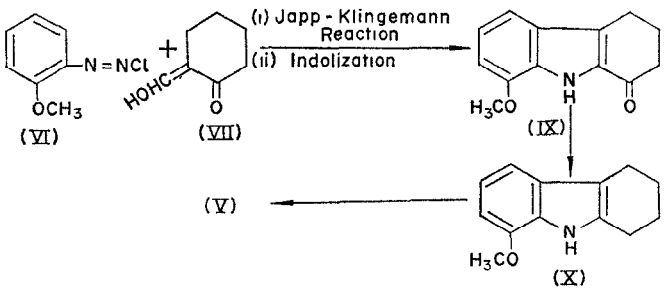
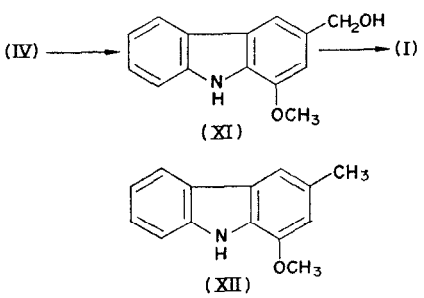
one methoxyl group and a carboxylic acid group. The IR spectrum (KBr) of (II) showed peaks at 3431 (—NH function), 1690 (—COOH group), 1635, 1613 and 1609 cm^{-1} (aromatic system with unsaturation). The UV spectrum of the compound showed absorption max at 235, 210 and 320 nm (log ϵ 4·51, 4·58, 3·92). On methylation with methyl iodide in presence of K_2CO_3, II furnished the *N*-methyl methyl ester (III), $C_{16}H_{15}NO_3$, m.p. 133°. With diazomethane, II furnished the methyl ester (IV), $C_{15}H_{13}NO_3$, m.p. 198° which on hydrolysis with 10% alcoholic KOH furnished II.

The nature of hydrogen functions of II was deduced from the NMR data of IV, since II was insoluble in the usual NMR solvents. The NMR spectrum of IV (DMSO, 60 mc) showed the presence of six aromatic protons, one of which was at low field (δ 8·15); three proton singlets of an aromatic methoxy group (δ 4·02) and of a carbomethoxy group (δ 4·07). The low field proton at δ 8·15 could be the proton α to the carbomethoxy group which is probably at 4-H.[5] The presence of a carbazole skeleton with an aromatic methoxy and a carboxylic acid group in II, is consistent with the physical data. The isolation of carbazole from II by zinc dust distillation confirms it has a carbazole skeleton.



Decarboxylation of II with $SbCl_3$ at 145–150° gave a compound (V), $C_{13}H_{11}NO$, m.p. 69°. This was identified as 1-methoxycarbazole by comparison with a synthetic specimen prepared from diazotized *O*-anisidine (VI) and 2-hydroxymethylene-*cyclo*-hexanone (VII) via compounds (VIII), (IX) and (X).[8] This proves that the methoxy group in II is at C-1. The proton at C-2 of (IV) like that of murrayanine does not show any upfield shift due to the presence of the methoxyl group at 1. But the proton at C-4 exhibits a down field shift due to vicinal-COOMe group.

The data, therefore, lead to the formulation of mukoeic acid as II. This has been confirmed by the conversion of IV to I via XI. On reduction with $LiAlH_4$ under mild condition,



[8] D. P. CHAKRABORTY, K. C. DAS, B. K. CHOUDHURY, *Phytochem.* **8**, 773 (1969).

IV furnished the alcohol XI, $C_{14}H_{13}NO_2$, m.p. 127° which on oxidation with active MnO_2 furnished I. Since murrayanine (I), on treatment with 10% alkali, remained unchanged, mukoeic acid was not an artifact of isolation.

The co-occurrence of 1-methoxy-3-methylcarbazole (XII),[2,9] murrayanine and mukoeic acid in *M. koenigii* suggests that aromatic *C*-methyl groups in the carbazole alkaloids of the Rutaceae may be oxidized *in vivo* to formyl and carboxylic acid groups.[10]

EXPERIMENTAL

Isolation of Mukoeic Acid (II)

The alcoholic extract of the petroleum defatted stem bark (1 kg) of *Murraya koenigii* Spreng., on removal of solvent, furnished a residue. This was taken up with ethyl acetate (500 ml) and extracted thrice with 1% aq. NaOH. The alkaline extract (500 ml), on acidification in the cold, was extracted with ethyl acetate. The ethyl acetate fraction was made HCl-free and distilled. The residue so obtained, was dissolved in benzene and chromatographed over silica gel. Eluents were collected in fractions of 50 ml each. The $CHCl_3$ eluents (fractions 15–20) furnished a yellowish oil. This, on keeping and repeated crystallizations from benzene and ethyl acetate, furnished mukoeic acid, m.p. 242° (yield 50 mg). Homogeneity of the compound was examined by TLC (R_f, 0·41 in benzene–ethyl acetate–formic acid (75:24:1); violet fluorescence under chromatolite, 2300 Å; the spot gives brick-red coloration when sprayed with picric acid). (Found: C, 69·57; H, 4·71; N, 5·91; OMe 12·76. $C_{14}H_{11}NO_3$ required C, 69·70; H, 4·60; N, 5·81; one OMe 12·81%.) The picrate of (II) melted at 225–228°. (Found: C, 50·96; H, 2·95; N, 12·06. $C_{20}H_{14}N_4O_{10}$ required: C, 51·07; H, 3·00; N, 11·91%.)

Methyl Ester of N-*Methyl Mukoeic Acid (III)*

Mukoeic acid (100 mg) in dry acetone (10 ml) and MeI (1 ml) in presence of dry K_2CO_3 was refluxed for 6 hr and then filtered. The solid residue obtained after the removal of the solvent on repeated crystallizations from a mixture of benzene and petroleum afforded needles, m.p. 133° (yield 75 mg). The compound was found homogeneous by TLC (R_f 0·76 in acetic acid–petrol, 1:5). (Found: C, 71·5; H, 5·30; N, 4·58. $C_{16}H_{15}NO_3$ required C, 71·36; H, 5·61; N, 5·20%.)

Methyl Ester of Mukoeic Acid (IV)

The methanolic solution (25 ml) of mukoeic acid (200 mg) kept in a refrigerator for 16 hr with CH_2N_2 when a residue was obtained after the removal of the solvent. The residue was washed with 1% aq. NaOH when a colourless solid, m.p. 187°, was obtained. On further crystallization from benzene, the methyl ester (IV) m.p. 198° (yield 160 mg) was obtained. The compound was homogeneous on TLC (R_f 0·79, in benzene–$CHCl_3$ (1:1); blue violet flourescence under chromatolite 2300 Å; with picric acid, the spot gave a brick red colour).

Hydrolysis of (IV): Regeneration of (II)

The compound IV (50 mg) was hydrolysed with 10% alc. KOH (5 ml) under reflux (5 hr) on a water bath. On the removal of the solvent and acidification, a precipitate identical with II (mixed m.p., TLC) was obtained.

Zinc Dust Distillation of Mukoeic Acid: Formation of Carbazole

Mukoeic acid (250 mg) was distilled with zinc dust (10 g).[3] From the neutral fraction of the reaction product, a solid, m.p. 225° was obtained after chromatography over alumina (7 g). The solid was identified with carbazole by comparison with a pure specimen (mixed m.p., UV, TLC).[1]

Decarboxylation of (II): Formation of (V)

An intimate mixture of II (100 mg) and $SbCl_3$ (1 g) was heated on an oil bath at 145–150° for 30 min. The reaction product in ether (250 ml) was washed with cold conc. HCl till free from excess of $SbCl_3$. The semi-solid mass obtained after the removal of ether was dissolved in benzene and chromatographed on silica gel (5 g). From the fractions eluted with petroleum and benzene, a colourless oil was obtained from which 1-methoxycarbazole (V) m.p. 69° (yield 50 mg) was obtained, identical with the synthetic product described below.

[9] Recently M. S. WADIA, N. C. L. of India, Poona, has informed us that he obtained the compound (XII) from *M. koenigii* Spreng.

[10] B. K. CHOUDHURY and D. P. CHAKRABORTY, *Phytochem.* **10**, 481 (1971).

Cyclohexane-1,2-dione-1-1′-methoxyphenylhydrazone (VIII)

Diazotized solution of *o*-anisidine (3 g) was added to 2-hydroxymethylenecyclohexanone (VII, 3·5 g) in methanol (35 ml) in presence of aq. NaOAc throughout 30 min. When an oily hydrazone layer separated. The $CHCl_3$ extract of the reaction product, on chromatographic separation on silica gel, gave an oily product from which crystals, m.p. 85° was obtained after several crystallizations from alcohol. (Found: C, 67·3; H, 6·87; N, 12·18; $C_{13}H_{16}N_2O_2$ required: C, 67·22; H, 6·94; N, 12·06%.)

1-*Methoxy-5,6,7,8-tetrahydro-8-oxo-carbazole (IX)*

Compound VIII (1·5 g) was boiled in a mixture of HOAc (12 ml) and conc. HCl (3 ml) for 3 min. On pouring the reaction mixture into iced water, a precipitate was obtained. On crystallizations from benzene, crystals m.p. 104° was obtained (yield 0·95 g). (Found: C, 72·39; H, 6 12; N, 6·48. $C_{13}H_{13}NO_2$ required: C, 72·54, H, 6·09; N, 6·51%.)

1-*Methoxy-5,6,7,8-tetrahydrocarbazole (X)*

Compound IX (0·5 g) in $(CH_2OH)_2$ (10 ml) was heated with N_2H_4, H_2O (99–100%, 0·75 g) and KOH (0·8 g) at 190° for 1 hr and up to 210° under reflux for 3 hr. On working up the reaction product, a solid, m.p. 186–188°, was obtained. (Found: C, 77·49; H, 7·48; N, 6·98: $C_{13}H_{15}NO$ required: C, 77·58; H, 7·51; N, 6·96%.)

1-*Methoxycarbazole*

Compound (X) was dehydrogenated with Pd/c (10%) in presence of *p*-cymene (5 ml) in a sealed tube. The oily product obtained after the removal of oil was purified and crystallized from a mixture of benzene and petroleum when a compound m.p. 69–70° was obtained (lit, m.p. 69°). (Found: C, 79·08; H, 5 58; N, 7·18. $C_{13}H_{11}NO$ required: C, 79·17; H, 5·62; N, 7·10%.)

$LiAlH_4$ Reduction of IV

The reaction mixture containing IV (150 mg) $LiAlH_4$ (250 mg) and tetrahydrofuran (20 ml) was stirred for 3 hr. An oily product isolated from the reaction products furnished, on repeated crystallizations from benzene and petroleum, colourless needles (75 mg), m.p. 127°, identical with compound XI[2] (mixed m.p., TLC).

Oxidation of Compound XI to Murrayanine

A solution of compound XI (50 mg) in CCl_4 with suspension of freshly prepared active MnO_2 (500 mg) was stirred for 5 hr and filtered. On removal of the solvent, a solid was obtained which afforded murrayanine,[2] m.p. 168° (mixed m.p., IR). (Found: C, 74·61; H, 4·86; N, 6·34. $C_{14}H_{11}NO_2$ required: C, 74·5; H, 4·92; N, 6·22%.)

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