Physical Sciences Section

Pakistan J. Sci. Ind. Res., Vol. 28, No. 1, February 1985

STUDIES IN THE NONTERPENOIDAL CONSTITUENTS OF *AZADIRACHTA INDICA*

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(Received November 11, 1984)

Nimbochalcin (a dihydrochalcone derivative) (1) and nimbocetin (a substituted aromatic ester) (2), two new constituents have been isolated from the aqueous fraction of the fruits of *Azadirachta indica*, along with known 5-hydroxymethyl furfural. The structures of these constituents have been established through spectral studies. In addition to these components, a glycoside of quercetin and two flanonoids, quercetin and *iso*rhamnetin, have been obtained from aqueous fraction of leaves.

INTRODUCTION

Azadirachta indica A. Juss. (syn. *Melia azadirachta* Linn), commonly known as neem, belongs to the Meliaceae family and is indigenous to the Indo-Pakistan subcontinent [1]. Its different parts are highly reputed for the treatment of various human ailments, particularly against the diseases of bacterial and fungal origin. Its leaves have been used in ulcers, eczema, jaundice and liver complaints, whereas its fruits are considered as purgative, emollient and anthelmintic [2, 3].

More recently it has been shown that nimbidin, an amorphous factor isolated by Siddiqui [4] from the mother liquors of nimbin and nimbinin in 1942, is anti-arthritic and anti-inflammatory in its action [5], whereas various other fractions have been found to have anti-tumour [6], antipyretic and anti-inflammatory properties [7].

RESULTS AND DISCUSSION

In view of the therapeutic importance of the various parts of neem tree, present work has been undertaken on the non-terpenoidal fractions of its leaves and ripe and unripe fruits. Following the isolation procedures described in the Experimental, the hydrolyzate of the water soluble fraction of the unripe, undried fruits afforded a dihydrochalcone derivative and a substituted aromatic ester named respectively as nimbochalcin (1) and nimbocetin (2), which have not so far been reported in literature. Nimbochalcin (1) may prove to be of therapeutic importance, as several chalcone and dihydrochalcone derivatives have been noted in literature to possess significant biological activities]8, 10]. The hydrolyzate of water soluble fraction of ripe fruits afforded a furfural derivative which has been identified as 5-hydroxymethyl furfural on the basis of spectral studies and comparison of ^{13}C-NMR chemical shifts with the published data [11]. Isolation of 5-hydroxymethyl furfural from natural sources as well as its formation through acidic dehydration of various sugars has been recorded in literature [12, 13]. Further, from the water soluble fraction of leaves, a major flavonoidal glycoside has been isolated which on hydrolysis yielded quercetin along with a sugar rhamnose. Acidic hydrolysis of its mother liquor gave two flavonoids, quercetin and *iso*rhamnetin, which have been identified on the basis of their chemical characteristics and physical data [14]. This is the first reported isolation of *iso*rhamnetin from any part of neem tree, while quercetin has been isolated earlier from its leaves [15]. Recently quercetin has been found to show anti-tumour-promoter activity [16].

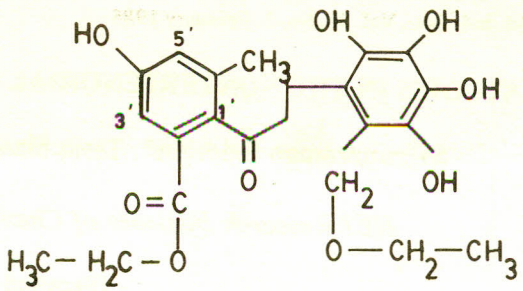
Nimbochalcin (1) was obtained as an oily residue and gave positive $FeCl_3$ test indicating the phenolic nature, while a negative Shinoda reaction [17] suggested that it does not have a flavonoidal skeleton. The mass spectrum showed a molecular ion peak at m/z 434.1567 corresponding to the molecular formula $C_{22}H_{26}O_9$. The ir spectrum showed bands at 3500 cm^{-1} (–OH), 1720 cm^{-1} (aryl ester), 1660 cm^{-1} (aryl ketone) and 3100, 1400-1600 (4 peaks), 870 cm^{-1} (aromatic ring). Its uv spectrum showed maxima at 227,278 and 328 nm. The appearance of an A_2B_2 system from δ 2.40-3.40 (α and β protons) in the ^{1}H-NMR spectrum indicated the presence of a dihydrochalcone skeleton [9] which was further supported by the absence of signals between δ 7.30-8.50 (AB quartet) and ca. δ 5.60 (X part of the ABX system) characteristic of chalcones and flavanones respectively [18]. It showed a five-proton broad signal extending from δ 4.80-5.40 for OH groups, while the signal for chelated hydroxyl group was not observed, indicating the absence of any hydroxyl function in

vicinity of the carbonyl group. A sharp singlet at δ 2.25 has been attributed to the methyl group on benzene ring while a three-proton triplet and a two-proton quartet at δ 1.30 and δ 4.15 respectively are due to ethyl ester group. A two-proton singlet observed at δ 4.58 ($\emptyset CH_2$-O-), a three-proton triplet at δ 1.25 (-O-CH_2-CH_3) and a two-proton quartet at δ 3.60 (-O-CH_2-CH_3) are consistent with the partial structure $\emptyset$-CH_2-O-CH_2-CH_3. A pair of doublets at δ 6.60 and δ 7.30 have been assigned to two aromatic protons with J = 2.5Hz, indicating that they are in *meta* position to each other. The absence of any other signal in the downfield region of the spectrum indicated that one of the two benzene rings is fully substituted. The appearance of fragments, resulting from the cleavage of the bond α and β to the carbonyl group at m/z 207.0646 and 221.0813 in the mass spectrum corresponding to the formulae $C_{11}H_{11}O_4$ and $C_{12}H_{13}O_4$ respectively, led to the placement of various functionalities in ring A and B as shown in structure (1).

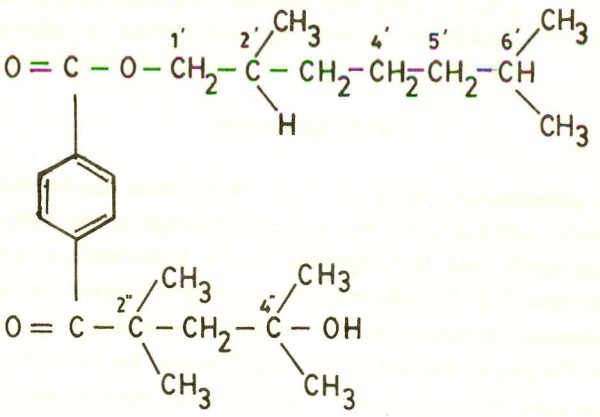
Of several possible substitution patterns of various functions, the one shown in (1) appears to be the most plausible on the basis of mass and ^{1}H-NMR data. However, lack of sufficient quantity of nimbochalcin precluded extensive chemical studies.

Nimbocetin (2) $C_{24}H_{38}O_4$, gave negative $FeCl_3$ and Shinoda tests indicating that it is not phenolic or flavonoidal in nature. Its ir spectrum showed important peaks at 3350 Cm^1 (–OH), 1720 cm^{-1} (aromatic ester), 1660 cm^{-1} (aromatic ketone), 3120, 1420-1595 (4 peaks) and 860 cm^{-1} (benzene ring) and 1380 cm^{-1} (gem dimethyls), while uv spectrum showed maxima at 213, 226 and 280 nm. It remained unreacted when treated with acetic anhydride/pyridine or with diazomethane indicating that the hydroxyl group is alcoholic in nature and located at tertiary carbon.

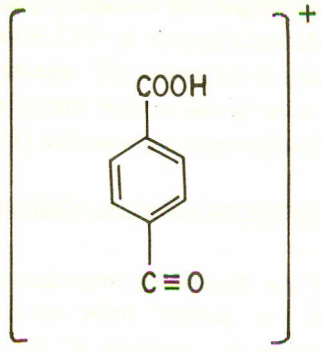
The ^{1}H-NMR spectrum showed a four protons AA′ BB′ apparently simple multiplet extending from δ 7.45 to δ 7.90 indicating that the carbonyl and ester groups are *para* substituted on the benzene ring. The appearance of diagnostic fragments at m/z 43, 57, 71, 85, 113 and 127 showed the presence of nine-carbon side chain with branching at C-2′ and C-6′; and the appearance of two-proton doublet at δ 4.20 (J = 6.25Hz, 1′-H) in the ^{1}H-NMR spectrum showed that this side chain is attached to the ester oxygen. On the other hand, a seven-carbon oxygenated side chain linked to the carbonyl function has been inferred from the significant ions at m/z 41 (59-18), 55 (73-18) and 97 (115-18) which also implied that the hydroxyl group is located at C-4″. The absence of any signal at ca. δ 2.60 in the ^{1}H-NMR spectrum led to the



1



2



ion 'a'

placement of two methyl groups at C-2″ which was further supported by a two-protons singlet of 3″-H at δ 1.30. The base peak at m/z 149.0238 corresponding to formula, $C_8H_5O_3$ which has been attributed to the ion 'a', conclusively demonstrated the presence of a benzoyloxy ketone. The two terminal secondary methyl groups appeared as a six-proton doublet at δ 0.85 (J = 6.5Hz) while the two terminal tertiary methyl groups resonated as three-proton singlets at δ 0.91 and δ 0.93. The methyl group at C-2′ was observed as a three-protons doublet at δ 0.96

(J = 6.5Hz), while the two methyl groups located at the carbon adjacent to the carbonyl function appeared as six-protons singlet at δ 1.24. On the basis of these findings, the structure of nimbocetin has been concluded as 4-(2″, 2″, 4″-trimethyl-4″-hydroxy pentan-1″ -one)-2′, 6′-dimethyl-heptyl -benzoate (2).

EXPERIMENTAL

General experimental procedure. Their spectra were taken on JASCO IRA-I spectrometer. The UV spectra were taken on Pye-Unicam SP-800 spectrometer. Mass spectra were determined on Finnigan MAT 112 and MAT 312 double focusing mass spectrometers connected to PDP 11/34 computer system. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker WP-100-SY FT-NMR spectrometer with TMS as internal reference. The purity of samples was checked on tlc (silica gel SIF-254) precoated aluminium cards.

Plant material. The plant materials examined in the present studies were collected in Karachi and a voucher specimen is deposited in the herbarium of the Botany Department of Karachi University.

Extraction of unripe fruits. Fresh, undried, unripe fruits (20 kg) were percolated at room temperature with ethanol. After removal of the solvent at reduced pressure, the thick liquidish mass was partitioned between water and ethyl acetate.

Nimbochalcin (1). 250 ml of the concentrated aqueous phase was acidified with 6% methanolic HCl (450 ml) to pH 1 and extracted out with ethyl acetate to remove the acidic components. The ethyl acetate layer was washed with saline which was taken with the aqueous phase, refluxed for 4 hr. and extracted out with ethyl acetate. The ethyl acetate layer was washed with saline, finally with water to remove the alcohol, dried, concentrated and repeatedly purified with ether and petroleum ether. The purified mixed solvent soluble fraction, on removal of the solvent yielded an oily residue (0.015% on dry weight basis) which showed a single spot on tlc, uv λ_{max} (log ε) (MeOH) 328 (3.98), 278 (4.78) and 227 (4.34) nm; IR ν_{max} ($CHCl_3$) 3500, 3100, 2800, 1720, 1660, 1400-1600 (4 peaks), 1340, 1070, 1000 and 870 cm^{-1}; $C_{22}H_{26}O_9$ (Calcd. 434.1576; found 434.1567); ms rel. intensities %) m/z 434 (1, M^+), 302 (2), 268 (2), 252 (6), 235 (16), 221 (100), 213 (2), 207 (18), 73 (40) and 59 (74); ^{1}H-NMR ($CDCl_3$) δ: 7.30 (1H, d, J = 2.5Hz, 3' –H), 6.60 (1H, d, J = 2.5Hz, 5' –H), 4.80-5.40 (5H, hump, 5–OH, ex.D_2O), 4.58 (2H, s, Ø -CH_2-O-), 4.15 (2H, q, J = 7.5Hz, Ø -C-O-CH_2-), 3.60 (2H, q, J = 7.5Hz, O-CH_2-CH_3), 2.40-3.40 (A_2B_2 pattern for *α* and *β* protons), 2.25 (3H, s, 6′-CH_3), 1.30 (3H, t, J = 7.5Hz, -C-O-CH_2-CH_2) and 1.25 (3H, t, J = 7.5Hz-O-CH_2-CH_3-).

Nimbocetin (2). In an alternative working, the main aqueous phase was concentrated to a thick mass, which was successively digested with acetone, ethanol and methanol. The insoluble residue was taken in 10% aqueous HCl and heated in water bath for 1 hr., cooled and extracted with ethyl acetate. The ethyl acetate layer after usual workup afforded a gum (yield 0.02% on dry weight basis) showing a homogeneous spot on tlc; uv λ_{max} (log ε) (MeOH) 280 (4.55), 226 (4.53) and 213 sh. nm; ir ν_{max} ($CHCl_3$) 3350, 3120, 2830, 1720, 1660, 1420-1595 (4 peaks), 1380, 1240, 1120, 1040 and 860 cm^{-1}; $C_{24}H_{38}O_4$ (Calcd. 390.2770, found 390.2791); fims (rel. intensity %) m/z 390 (36); eims (rel.intensities %) m/z 390 (0.21, M^+), 245 (0.1), 191 (0.2), 150 (10), 149 (100), 141 (1.2), 127 (1), 126 (0.6), 125 (1), 120 (2), 114 (1.1), 113 (13), 97 (3), 85 (4), 76 (3), 73 (0.3), 71 (30), 65 (2), 59 (0.2), 57 (45), 55 (24), 43 (39), and 41 (24); ^{1}H-NMR ($CDCl_3$) δ: 7.45-7.90 (AA′ BB′ pattern for four aromatic protons), 4.20 (2H, d, J = 6.25Hz, 1′-H), 1.30 (2H, s, 3″-H), 1.24 (6H, s, 2″-CH_3x2), 0.96 (3H, d, J = 6.5Hz, 2′-CH_3), 0.91 and 0.93 (6H, 2xs, 5″-H and 4″-CH_3) and 0.85 (6H, d, J = 6.5Hz, 7′ –H and 6′ –CH_3).

Extraction of ripe fruits. Fresh, undried, ripe fruits (10 kg) were percolated at room temperature with ethanol. The ethanolic extract was concentrated at reduced pressure, partitioned between water and ethyl acetate, and the aqueous phase was again concentrated at reduced presure.

5-Hydroxymethyl furfural. The thickish aqueous phase was acidified with 6% methanolic HCl to pH-1 and extracted out with ethyl acetate. The ethyl acetate layer was washed with saline which was taken with aqueous phase and refluxed on the water bath for 1 hr. It was extracted out with ethyl acetate, washed, dried and freed of the solvent and the oily residue was divided into ether soluble and insoluble fractions. Thick layer chromatography of ether soluble fraction in benzene-ethyl acetate (7:3) afforded three compounds, one of which obtained as a light yellow oil (0.69% on dry weight basis) has been identified as 5-hydroxymethyl furfural, uv λ_{max} (MeOH) 205, 227 and 280 nm; ir ν_{max} ($CHCl_3$) 3350 (-OH), 3100 (furan), 2820 (aromatic–CH str.), 2700 (aldehydic-CH str.), 1670 (aldehydic carbonyl) and 1500 cm^{-1} (furan); $C_6H_6O_3$ (Calcd. 126.0317; found 126.0313); ms, (rel. intensities %) m/z 126 (48, M^+), 109 (94), 97 (98), 81

(46), 69 (78) and 57 (100); ^{1}H-NMR ($CDCl_3$) δ: 9.35 (1H, s, CHO), 7.13 (1H, d, J = 3.5Hz, 3-H). 7.09 (1H, d, J = 3.5Hz, 4-H), 4.53 (2H, s, -O-CH_2) and 4.33 (1H, br. s, -OH); ^{13}C-NMR ($CDCl_3$) δ: 177.58 (CHO), 160.92 (C-5), 151.40 (C-2), 123.63 (C-3), 109.50 (C-4) and 56.38 (-CH_2-O). The structures of other two compounds are under investigation.

Extraction of leaves. 40 kg. of fresh, undried leaves were percolated with ethanol at room temperature. The alcoholic extract was freed of the solvent under reduced pressure and partitioned between ethyl acetate and water.

Isolation of quercetin glycoside. On concentrating the main aqueous phase and keeping it at room temperature for several days the glycoside of quercetin was deposited as crystalline yellow mass. It was filtered, washed with water and recrystallized from methanol-water (2:1) when flowers of needles of the glycoside were obtained melting at 238-45° (yield 0.45% on dry weight basis).

Hydrolysis of the glycoside. 1 g. of the glycoside was heated with 5% ethanolic HCl for 30 min. and extracted with ethyl acetate. The ethyl acetate layer on usual workup afforded a crystalline mass which on recrystallization from moist ethyl acetate formed fine yellow needles melting at 250-52° which was identified as quercetin from its ir and mass spectra and by comparison of its uv and ^{1}H-NMR spectral data with those reported in literature [14]. The aqueous phase containing sugar was concentrated and analyzed by paper chromatography using solvent system *n*-butanol-ethanol-water (4:1:2.5) and found to contain rhamnose when compared with the standard sugar.

Isorhamnetin and quercetin. A portion (200 ml) of the mother liquor of the quercetin glycoside was acidified with 3% aqueous HCl to pH 1 and extracted out with ethyl acetate to remove acidic components. The aqueous acidic phase was heated on the water bath for 1 hr., cooled and extracted out with ethyl acetate. After usual working the ethyl acetate layer was concentrated and left at room temperature with the addition of a few drops of water. Quercetin was obtained through slow crystallization as yellow fine needles, mp 250-51° . The mother liquor yielded *iso*rhamnetin, through thick layer chromatography, which formed yellow needles, on recrystallization from ethyl acetate-methanol (2:1), mp 273-275°. Both these flavonoids were identified from their ir and mass spectra and comparison of their uv and ^{1}H-NMR with the reported data (14).

Acknowledgement. One of us (Tariq Mahmood) wishes to express grateful thanks to Hamdard Foundation of Pakistan for provision of research fellowship in the course of this work.

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