BHUBANESWIN: A NEW BICOUMARIN

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Abstract- The new bicoumarin bhubaneswin (3) has been obtained from Boenninghausenia albiflora Reichb. ex Meissner (Rutaceae).

Boenninghausenia albiflora Reichb. ex Meissner (Rutaceae) is a slender, erect, perennial rooted herb found notably in the temperate regions of the Himalayas at an altitude of 1,500 to 2,000 m. A variety of new and known coumarins have been obtained from this source, all derived biogenetically from 7-hydroxycoumarin (umbelliferone).[1] These include xanthyletin,[2,3] 3-(1,1-dimethylallyl)-xanthyletin,[4] bergapten,[2,3] isopimpinellin,[2] (-)-nodakenetin acetate,[2] xanthotoxin,[2] daphnetin-8-methyl ether,[2] angenomalin,[5] micropubscin,[5] angelical,[6] suberenon,[6] (E) and (Z)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2H-1-benzopyran-2-one,[3] rutamarin (chalepin acetate),[7] and chalepensin.[3] Two dimeric coumarins were also known to occur in B. albiflora. These are daphnoretin (1)[6] and matsukaze-lactone (2),[8] the latter not known to be present in any other plant.

Presently, a reinvestigation particularly focused on the bicoumarins of B. albiflora supplied again matsukaze-lactone (2), as well as the new dimer bhubaneswin (3). Bhubaneswin is phenolic, gives a yellow color in alkali, and fluoresces under UV light. Its molecular composition, $C_{19}H_{12}O_6$, as determined by mass spectroscopy, differs from that of the non-phenolic matsukaze-lactone (2), $C_{20}H_{14}O_6$, by one carbon and two hydrogens.

The similarity between the two dimers was brought to the fore by the fact that they had nearly identical UV spectra (Experimental). This similarity was further underscored by the ^1H NMR spectra of the two compounds. The $CDCl_3$ ^1H NMR spectrum of matsukaze-lactone is summarized around expression 2. The spectra for bhubaneswin in acetone-d_6 and in $CDCl_3$ are presented around expressions 3 and 3A, respectively.

The proton spectra of the new dimer 3 differ from that of 2 mainly by the absence of one of the

methoxyl singlets. All the spectra include two aromatic protons, each as doublets, representing H-5 and H-6, as well as two one-proton aromatic singlets due to H-5' and H-8'. It is thus evident that bhubaneswin is a monophenolic analog of matsukaze-lactone (2); and indeed O-methylation of bhubaneswin (3) using dimethyl sulfate gave rise to 2.

In order to differentiate between structure 3 and alternate expression 4 for bhubaneswin, the dimer was subjected to a specific nuclear magnetic resonance nuclear Overhauser enhancement difference study (NMR NOEDS)[9] in the solvent acetone-d_6, the result of which is indicated in expression 3. Irradiation of the methoxyl singlet at δ 3.87 led to a 33% increase in the area of the δ 7.07 singlet, while irradiation of the δ 7.07 absorption gave a 3.1% increase in the area of the δ 3.87 singlet. It should be noted here that reciprocal NOE's for two neighboring protons or sets of protons are not necessarily equal, but depend on the magnitude of the relaxation times T_1. Thus, irradiation of a fast relaxing proton close to a slow relaxing one will result in a larger NOE for the slow relaxing proton than when the reverse operation is carried out.[9] Structure 3 must, therefore, represent bhubaneswin.

Further support for structure 3 was obtained by acetylation of bhubaneswin to provide monoacetate 5. The most salient feature of the $CDCl_3$ NMR spectrum of this derivative was that the two-proton aromatic doublet of doublets due to H-5 and H-6 had now moved appreciably downfield to δ 7.56 and 7.15, respectively. This reflects the fact that O-acetylation occurred at a phenolic function originally present in ring A, rather than in ring A', as required by expression 3 for bhubaneswin.

Bhubaneswin (3) and matsukaze-lactone (2) should be juxtaposed by the known dimer 6, named bicoumol, and found in ladino clover.[10] The biogenetic sequence thus appears to start with the oxidative dimerization of 7-hydroxycoumarin to yield bicoumol (6),[8,10] whose in vivo O-methylation using S-adenosylmethionine leads to bhubaneswin (3). Further O-methylation then furnishes matsukaze-lactone (2).

Known monomeric coumarins reisolated in the course of the present study include 3-(1,1-dimethylallyl)-xanthyletin, rutamarin and bergapten.

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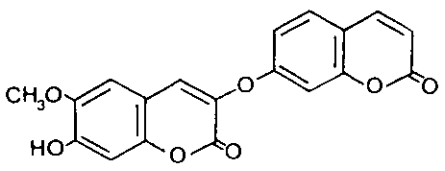

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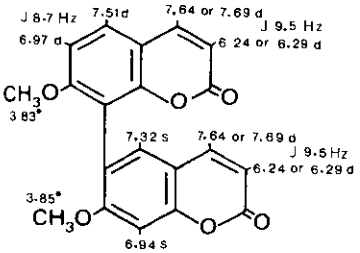

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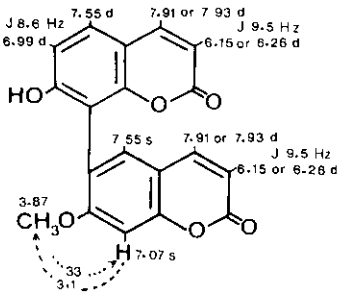

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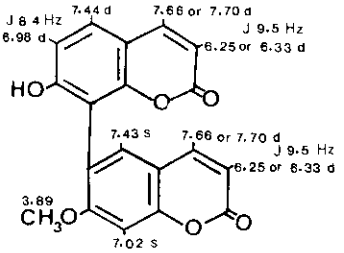
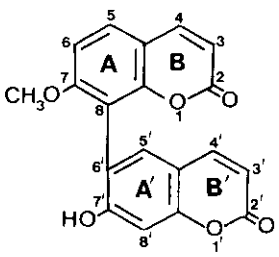


3A



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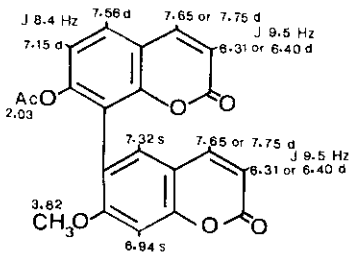

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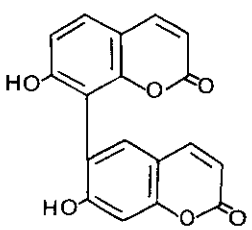

6

Chemical shifts with identical superscripts are interchangeable.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker 200 or 360 MHz spectrometer, and ^{13}C spectra were collected on a Bruker 50.32 MHz instrument. NOE's were determined by use of FT NOE difference spectroscopy. Four readings were acquired with the decoupler set exactly on a given resonance, and four readings with the decoupler off-resonance were then subtracted. This procedure was repeated until adequate signal to noise ratios were achieved.[9]

Isolation Procedure: The whole plant (4.7 kg, dry) was collected in the western Himalayas during June-July, 1981. The milled, air dried, plant was extracted with hot hexane. The solvent was evaporated and the residue kept in a refrigerator overnight. The oily mass that separated was filtered and washed with hexane. The filtrate was concentrated (27 g), and placed on a silica gel column (300 g). Elution was first with hexane, and then with hexane mixed with increasing amounts of ethyl acetate.

The fraction eluted with hexane furnished a solid which on further recrystallization from hexane supplied colorless crystals of 3-(1,1-dimethylallyl)xanthyletin, 93 mg, mp 97-98° C ; lit.[4] mp 98-99° C (acetone-petroleum ether)

Elution with hexane-ethyl acetate (94:6) furnished rutamarin, 41 mg, mp 105-106° C.[1] This compound was followed by bergapten, 83 mg, mp 188° C (MeOH); lit.[2] mp 188-191° C (pet. ether-$CHCl_3$).

Following the hexane extraction, the powdered plant material was extracted with cold chloroform. The extract (96 g) was placed on a silica gel column (600 g). Elution was first with hexane, followed by hexane-benzene mixtures, benzene, and finally benzene-ethyl acetate. Benzene elution supplied additional amounts of bergapten. From the benzene-ethyl acetate (9:1) elution, colorless cubes of matsukaze-lactone (2) were obtained, 85 mg, mp 266-267° C ($CHCl_3$); lit.[8] mp 267-268°C (acetone); which showed a blue fluorescence under UV light. Finally, the polar fraction, eluted with benzene-ethyl acetate (9:2) furnished colorless needles of bhubaneswin (3) (54 mg), mp 320°C (acetone-$CHCl_3$ 1:1).

Matsukaze-lactone (2): λ max (MeOH) 209, 324 nm (log ε 3.94, 3.80). ^{13}C NMR ($CDCl_3$) in ppm 56.2 (OCH_3), 56.3 (OCH_3), 99.6 (C-8'), 107.7 (C-6), 112.3 (C-8)*, 113.0 (C-4a')*, 113.1 (C-3')**, 113.4 (C-3)**, 113.8 (C-4a)*, 117.9 (C-6'), 128.6 (C-5'), 130.8 (C-5), 143.5 (C-4)#, 143.6 (C-4')#, 152.8 (C-8a)##, 156.0 (C-8a')##, 160.2 (C-2)###, 160.7 (C-2')###, 160.8 (C-7)###, 161.2 (C-7')###.

Bhubaneswin (3): ν max KBr 3250, 1725, 1680, 1600, 1205, 1145, 1100, 1005, 910, 825 cm^{-1}; λ max (MeOH) 211, 326 nm (log ε 4.08, 3.95); λ max (MeOH-OH^-) 213, 356, 375 nm (log ε 4.15, 3.80, 3.81). ^{13}C NMR (DMSO-d_6) in ppm 56.2 (C-7' OCH_3), 99.4 (C-8'), 111.0 (C-6), 111.2 (C-8)*, 111.4 (C-4a')*, 111.9 (C-4a)*, 112.5 (C-3')**, 112.7 (C-3)**, 118.2 (C-6'), 128.6 (C-5'), 131.1

(C-5), 144.1 (C-4')***, 144.7 (C-4)***, 152.9 (C-8a)$^{\#}$, 155.3 (C-8a')$^{\#}$, 159.0 (C-7)$^{\#\#}$, 160.1 (C-2)$^{\#\#}$, 160.2 (C-2')$^{\#\#}$, 160.6 (C-7')$^{\#\#}$; ms m/z 336 (M^+) (100), 319 (12), 305 (35), 291 (18), 277 (13), 265 (17), 249 (11), 163 (7), 152 (11).

O-Methylation of 3: Bhubaneswin (40 mg), anhydrous potassium carbonate (80 mg) and dimethyl sulfate (0.2 mL) in dry acetone (15 mL), were refluxed for 3 h. Removal of the solvent gave a residue which was poured into water. Recrystallization of the colorless crystals provided 2 (23 mg) spectrally identical with matsukaze-lactone, mp 266-267° C ($CHCl_3$).

O-Acetylation of 3: Bhubaneswin (25 mg) was dissolved in pyridine (2 drops) and acetic anhydride (2 mL) was added. The solution was left at room temp overnight. Work-up led to monoacetate 5, $C_{21}H_{14}O_7$, 12 mg, mp 275° C (CH_2Cl_2).

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