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A PRENYLATED COUMARIN WITH ANTIMICROBIAL ACTIVITY FROM *HAPLOPAPPUS MULTIFOLIUS*

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Key Word Index—*Haplopappus multifolius*; Compositae; coumarins; prenyletin; haplopinol; aesculetin.

Abstract—The coumarins prenyletin, haplopinol and aesculetin were shown to be responsible for the antimicrobial activity of *Haplopappus multifolius*. The structure of the new coumarin haplopinol was established by analysis of its spectral data.

INTRODUCTION

In a large-scale study of antimicrobial activity in higher plants [1], it was found that an alcoholic extract of leaves of *Haplopappus multifolius* Reiche had reproducible activity, *in vitro*, against *Staphylococcus aureus*. The aerial parts of this plant have been used in folk medicine but there are no reports on its chemical composition.

RESULTS AND DISCUSSION

The benzene-soluble extract of dried leaves was fractionated by CC on Si gel to yield prenyletin (**1**). The ethyl-acetate-soluble extract, similarly fractionated, gave haplopinol (**2**) and aesculetin (**3**).

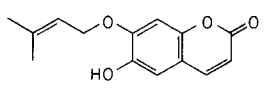
Prenyletin (**1**) mp 140–143°, M^+ 246 corresponding to $C_{14}H_{14}O_4$ has been previously isolated [2, 3]. Prenyletin behaves as a 6-hydroxycoumarin since its UV spectrum, λ_{max} 232, 250 and 297 nm is changed by base in a characteristic fashion, the new bands being at 254, 315 and 403 nm, almost identical to the behaviour of 6-hydroxy-7-methoxycoumarin and different from scopoletin, which, in base, absorbs at 242 and 400 nm [2–4]. This coumarin was identified by its spectroscopic data and by direct comparison with an authentic sample.

Haplopinol (**2**) mp 195–198° was obtained from the ethyl acetate extract. This compound had M^+ 262 corresponding to $C_{14}H_{14}O_5$ and its UV spectrum behaves almost identically with the spectrum obtained from prenyletin (**1**) taken directly and after basic treatment. The IR spectrum had bands at 3480 (hydroxyl), 1690 (ketone), 1635 (double band) and 1580 cm^{-1} (aromaticity). The ^1H NMR spectrum of haplopinol had signals at δ 1.78 for a methyl group, δ 4.04 for the methylene bearing the hydroxyl group, δ 4.67 as a doublet ($J = 7.5$ Hz) for the methylene group of the side-chain, δ 5.80 a triplet ($J = 7.5$ Hz) due to the olefinic proton, δ 6.18 and 7.70 ($J = 9.5$ Hz) as a double doublet typical of H-3 and H-4 of a coumarin and at δ 6.90 and 7.10 the aromatic protons H-5 and H-3. The MS was easily rationalized for the expected fragmentation pattern of haplopinol [5, 6].

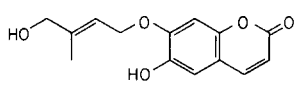
Haplopinol on acetylation afforded an acetate, mp 125–127°, M^+ 346 corresponding to $C_{18}H_{18}O_7$. The IR spectrum of this compound gave peaks for acetate, ketone and aromatic absorption and its ^1H NMR spectrum had signals at δ 2.10 and 2.30 for two acetates, one methyl group, two aromatic protons and the typical double doublet for H-3 and H-4 of a coumarin. On the basis of the above data, structure (**2**) is proposed for haplopinol. The (*E*)-configuration about the side-chain olefinic bond follows from the observed chemical shift of the CH_2OH group, which occurs at δ 4.04. In related compounds, such as trichoclin [7], this occurs in the range 3.91–4.03 whereas, in related compounds bearing the (*Z*)-configuration, such as arnottinin [8], the range of values is 4.34–4.42. Aesculetin was isolated from the ethyl acetate extract and was identified by comparison with an authentic sample.

Biological activity

The ethanolic extract of *Haplopappus multifolius* was found to be active *in vitro* against *Staphylococcus aureus* (ATCC 6538P), *Sarcina lutea* (UCL 51)



Prenyletin, 1



Haplopinol, 2

and *Escherichia coli* (UCCS1) as well as some of its subsequent fractions [1].

The antibacterial activity of aesculetin upon different micro-organisms has also been tested by Jurd *et al.* [9]. These authors have reported that it exhibits a broad-spectrum antibacterial activity, since adequate inhibition was found to produced upon strains of both Gram-positive and Gram-negative bacteria. However, no quantitative assays were performed in order to further investigate its bacteriostatic or bacteriocidal activity. Dadák and Hŏdák have also investigated the antibacterial activity of some other natural coumarins upon Gram-positive microorganisms [10]. They found activity in 6 of 10 compounds tested with paper discs at 5×10^{-3} M concentrations.

The pure coumarins were tested and their minimal inhibitory and minimal bactericidal concentrations [11] were determined. It was found (Table 1) that the activity of these compounds required the presence of the aromatic hydroxyl group and prenylated chain; their acetates were found to be inactive.

EXPERIMENTAL

General procedures. Mps are uncorrected. UV spectra were obtained in MeOH and IR spectra with Nujol. ^{1}H NMR spectra were recorded on a Varian HA 100 spectrometer using tetramethylsilane as internal reference. Si gel G (Merck) was used for the plates.

Extraction. The leaves and stems of *Haplopappus multifolius* were collected in September near Los Andes, V Región, Chile. Eleven kg of the dried leaves were percolated in 50% EtOH until exhaustion. Concentration of the combined eluates under red. pres. at 50–60° gave 850 g of crude extract.

Isolation of prenyletin (**1**). The total EtOH extract was treated with benzene and gave a dark benzene-soluble residue (6 g). This residue was fractionated by gradient elution column over SiO_2. The column eluted with increasing proportion of EtOAc afforded prenyletin (75 mg, 0.007%) mp 140–143°, UV λ_{max}^{MeOH} nm: 232, 297, 350; UV $\lambda_{max}^{MeOH+NaOH}$ nm: 254, 315, 403; MS *m/z*: 246 (M^+) corresponding to $C_{14}H_{14}O_4$. Comparison with authentic prenyletin showed them to be identical.

Isolation of haplopinol (**2**). The original benzene extract was further treated with EtOAc to yield a soluble fraction (50 g). The EtOAc extract was fractionated by chromatography on SiO_2. Elution with C_6H_6–EtOAc (3:2) yielded haplopinol (55 mg, 0.005%) mp 195–198° (EtOAc), λ_{max}^{MeOH} nm: 212 (ε 19,600), 230 (ε 17,600) 255 (ε 6600), 295 (ε 7800), 348 (ε 12,600); $\lambda_{max}^{MeOH+NaOH}$ nm: 255, 315, 403; ν_{max}^{Nujol} cm^{-1}: 3480, 2880, 1690, 1635, 1580, 1470, 1400, 1295, 1170, 1010, 880, 825; ^{1}H NMR δ ($CDCl_3$): 1.78 (3H, *s*, CH_3), 4.04 (2H, *s*, CH_2OH), 4.67 (2H, *d*, $J = 7.5$ Hz, OCH_2), 5.80 (1H, *t*, $J = 7.5$ Hz, C=CH), 6.18 (1H, *d*, $J = 9.5$ Hz, H-3), 6.90 (1H, *s*, H-5), 7.10 (1H, *s*, H-8), 7.70 (1H, *d*, $J = 9.5$ Hz, H-4); MS *m/z* 262(19) M^+, 244 (25), 178 (100), 60 (30), 44 (85).

Haplopinol acetate. 20 mg of haplopinol were treated with pyridine and Ac_2O at room temp. to yield upon crystallization, the diacetate, mp 125–127°; ν_{max}^{Nujol} cm^{-1}: 2970, 1700, 1630, 1580, 1470, 1385, 1290, 1270, 1250, 1145; ^{1}H NMR δ ($CDCl_3$) 1.78 (3H, *s*, CH_3), 2.10 (3H, *s*, CH_3CO), 2.30 (3H, *s*, CH_3CO), 4.52 (2H, *s*, CH_2OAc), 4.67 (2H, *d*, $J = 7.5$ Hz, $O\text{-}CH_2$), 5.60 (1H, *t*, $J = 7.5$ Hz, C = CH), 6.30 (1H, *d*, $J = 9.5$ Hz, H-3), 6.80 (1H, *s*, H-5), 7.20 (1H, *s*, H-8), 7.60 (1H, *d*, $J = 9.5$ Hz, H-4).

Isolation of aesculetin. This column also yielded aesculetin (43 mg, 0.004%) identified by direct comparison with an authentic sample. This compound was further characterized through its acetate.

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Table 1. Minimal inhibitory and minimal bactericidal concentration of the coumarins isolated

Compound tested	*S. aureus* MIC*	*S. aureus* MBC†	*E. coli* MIC*	*E. coli* MBC†	*S. lutea* MIC*	*S. lutea* MBC†
Prenyletin	250‡	250	—	—	250	500
Prenyletin acetate	—	—	—	—	—	—
Haplopinol	500	500	1000	1000	—	—
Haplopinol acetate	—	—	—	—	—	—
Aesculetin	500	1000	500	500	250	500
Aesculetin acetate	—	—	—	—	—	—

*Minimal inhibitory concentration.
†Minimal bactericidal concentration.
‡μg/ml.
—Not tested (no activity found in qualitative test).

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PHENANTHRENE DERIVATIVES FROM *ARISTOLOCHIA ARGENTINA*

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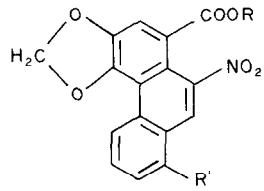
Key Word Index—*Aristolochia argentina*; Aristolochiaceae; aristolochic acid; aristolochic acid methyl esters.

Abstract—Aristolochic acid Ia, aristolochic acid I methyl ester and aristolochic acid II methyl ester were identified in the roots of *Aristolochia argentina*.

A previous investigation[1] established the occurrence in the roots of *Aristolochia argentina* of six aristolochic acids. Three new compounds, biogenetically related to aristolochic acid I (**1**) and aristolochic acid II (**2**), are now reported from the same source, aristolochic acid Ia (**3**), aristolochic acid I methyl ester (**4**) and aristolochic acid II methyl ester (**5**). Two, **3** and **5**, are reported for the first time as natural plant products.

Aristolochic acid Ia is a minor component, *ca* 0.8%, of the fraction of phenolic aristolochic acids and could be characterized therefrom as its derivatives **4** and **6**. The roots of *A. argentina* contain 0.7 μg/g (dry wt) of this acid. Aristolochic acid Ia has been also found by Rothschild *et al.*[2] to occur in *Zerynthia polyxena*, a butterfly whose larvae feed on *Aristolochia clematitis*.

Aristolochic acid I methyl ester and aristolochic acid II methyl ester were found in the petrol extract. Their content in the roots amounts to 4.6 and 0.03 μg/g (dry wt), respectively. The former (**4**) was previously isolated from *Aristolochia indica* by Pakrashi *et al.*[3].



1 R = H; R' = OMe
2 R = H; R' = H
3 R = H; R' = OH
4 R = Me; R' = OMe
5 R = Me; R' = H
6 R = Et; R' = OEt

EXPERIMENTAL

Dried roots of *A. argentina*, collected near Villa Allende (Córdoba, Argentina) in January 1974, were extracted with boiling petrol and EtOH as described earlier [1]. TLC separations were carried out with the following systems: (1) Al_2O_3–C_6H_6; (2) Si gel–C_6H_6 (two developments).

Aristolochic acid I methyl ester (**4**) *and aristolochic acid II methyl ester* (**5**). The petrol extract from 23 kg dried roots