Using GC conditions (B), 4-hydroxy-β-damascone (*ca* 0.02% of the oil) eluted at 40.57 min, bracketed by 3-hydroxy-8,9-dihydro-β-damascone, 38.40 min and the major isomer of 3-hydroxy-β-damascone, 43.23 min.

4-Hydroxy-β-damascone derived from the β-damascone 'ageing' expt co-chromatographed (R_t 78.81 min) with its counterpart in the tobacco subfraction using GC conditions (C).

'Ageing' of β-damascone. Samples of pure authentic β-damascone and H_2O were exposed to air and daylight for 6 months in flat soda glass bottles after which the β-damascone content was *ca* 10%. Alternatively 'accelerated ageing' was done by circulating an O_2 satd H_2O–β-damascone mixture in a thin film photoreactor (Applied Photophysics Ltd.) while irradiating with a white light spectrum lamp (Thorn 15W 'Northlight'). β-Damascone content was reduced to *ca* 10% in 1 month.

Isolation of **5** *and* **6** *from 'aged' β-damascone.* Distillation gave a fraction boiling 102–107°/0.1 mm Hg containing 96% **5** and **6**. Prep. HPLC, conditions (A), fractions monitored by GC conditions (A), gave **6** as white crystals, mp 66–69° uncorr. (hexane), lit. [7] 68–69°. Combined fractions containing **5** (60%) were rechromatographed, conditions (B), to give a clear, viscous, pale yellow oil. MS (probe) 70 eV m/z (rel. int.): 208.1450 $[M]^+$ (16), $C_{13}H_{20}O_2$ requires 208.1463, 193 $[M-Me]^+$ (22); 190.1394 $[M-H_2O]^+$ (5), $C_{13}H_{18}O$ requires 190.1358; 175 (12); 165 (5); 139.1125 $[M-C_4H_5O]^+$ (72), $C_9H_{15}O$ requires 139.1123; 69 (100); 43 (47); 41 (51). ^1H NMR (100 MHz, $CDCl_3$): δ1.05 (6H, *s*, Me_2C), 1.3–2.0 (4H, *m*, C-2 and C-3), 1.66 (3H, *s*, C-11), 1.88 (H, *s*, exchangeable D_2O, OH), 1.93 (3H, *dd*, $J_9 = 6.8$ Hz, $J_8 = 1.5$ Hz, C-10), 4.03 (H, *t*, $J_3 = 5$ Hz, C-4), 6.16 (H, *dq*, $J_9 = 16$ Hz, $J_{10} = 1.5$ Hz, C-8), 6.84 (H, *dq*, $J_8 = 16$ Hz, $J_{10} = 6.8$ Hz, C-9). IR ν_{max} cm^{-1}: 3449 vs (O–H), 1695, 1661 vs (C=O), 1456 s, 1389 and 1375 (Me_2C), 1302 s, 1258, 1238, 1180, 1084, 1029, 978 s (*trans*-CH=CH–), 935.

Dehydration of 4-hydroxy-β-damascone. Compound **5** (10 mg), *p*-toluenesulphonic acid (trace), and $CHCl_3$ (0.5 ml) were heated to 60° for 12 hr and analysed by GC/MS (conditions B). No **3** remained. The volatile products consisted of *ca* equal amounts of **1** and a compound of MW 236 thought to be the formate ester of **5**. MS (GC) 70 eV m/z (rel. int.): 236 $[M]^+$ (37), 221 $[M-15]^+$ (11), 207 $[M-29]^+$ (12), 167 $[M-69]^+$ (49), 139 (42), 69 (100), 43 (30), 41 (44).

Oxidation of 4-hydroxy-β-damascone. Compound **5** in Et_2O was stirred at 20° with nickel peroxide [8] until no **5** remained. Analysis (IR, GC/MS conditions B) showed **6** as the only volatile product.

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NAPHTHAZARINS FROM *ARNEBIA HISPIDISSIMA*

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Key Word Index—*Arnebia hispidissima*; Boraginaceae; isohexenylnaphthazarins; arnebin-7; alkannin acetate; alkannin isovalerate; alkannin; β-sitosterol; alkannin β-hydroxyisovalerate.

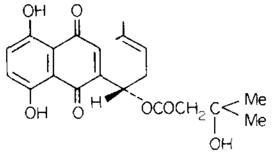
Abstract—Besides arnebin-7, alkannin acetate, alkannin isovalerate, alkannin and β-sitosterol, a new isohexenylnaphthazarin, alkannin β-hydroxyisovalerate, has been isolated from the roots of *Arnebia hispidissima*.

INTRODUCTION

Arnebia hispidissima DC., a shrub which grows wild in west Rajasthan, is one of the four species of the genus (Boraginaceae) occurring in northern India. The pigment from the roots is permitted for use in foodstuff by the Government of India [1]. Shikalkin [(±)-alkannin] has previously been reported as a constituent of the roots [2]. The growing importance of isohexenylnaphthazarins as potential anticancer agents [3, 4] prompted us to reinvestigate the roots. We now wish to report the isolation and structure elucidation of a new isohexenylnaphthazarin, alkannin β-hydroxyisovalerate, in addition to arnebin-7, alkannin isovalerate, alkannin acetate, alkannin and β-sitosterol.

RESULTS AND DISCUSSION

Alkannin β-hydroxyisovalerate (**1**) was isolated as violet-red needles, M^+ at *m/z* 388, $C_{21}H_{24}O_7$, mp 91–92° (uncorr.), $[\alpha]_D^{22}$ +86° (EtOH; *c*0.017). Its UV spectrum λ_{max}^{MeOH} nm (log ε): 276 (4.33), 488 (3.09), 525 (3.94) and 563 (3.77) indicated it to be naphthazarin. This was confirmed by a band at 1606 cm^{-1} in the IR spectrum [5], an alkali shift in the UV spectrum and a characteristic triplet absoption in the visible range [6] on addition of aluminium chloride to an ethanolic solution of **1**. Further support was provided by its ^1H NMR spectrum which showed two sharp singlets at δ12.63 and 12.46, D_2O exchangeable, for the two chelated phenolic hydroxyl protons and singlets at δ7.02 and at 7.2 for one proton on the quinonoid ring and two equivalent aromatic protons on the benzene nucleus, respectively.



I

In addition to above-mentioned signals, the ^1H NMR spectrum showed a sharp singlet (6H) at δ1.34 [methyl protons, $-C(OH)Me_2$], two singlets (3H each) at δ1.61 and 1.71 [methyl protons, $-CH=CMe_2$], a singlet (2H) at δ2.6 [methylene protons, $-CH_2C(OH)Me_2$] and a multiplet (2H) at δ2.58 for allylmethylene protons, the latter partially overlapping with the singlet of the other methylene protons. Apart from these resonances the spectrum had a broad singlet (1H, D_2O exchangeable) at δ3.16, a multiplet (1H) at δ5.14 and a triplet (1H) at δ6.1 assignable to hydroxyl, vinyl and methine protons, respectively.

Shikonin β-hydroxyisovalerate, $[\alpha]_{600}^{15}$ −108° (EtOH), isolated earlier from *Lithospermum erythrorhizon* [7], is indistinguishable from **1** by IR and NMR spectra. However, **1** is dextrorotatory $[\alpha]_D^{22}$ +86° (EtOH; *c*0.017). Thus the new naphthazarin is alkannin β-hydroxyisovalerate. Further confirmation for the assigned structure was obtained by alkaline hydrolysis of **1** (2% NaOH at room temperature, N_2 atmosphere) affording alkannin and β-hydroxyisovaleric acid.

Repeated CC of the hexane extract afforded four known naphthazarins and a sterol. Of these, alkannin and β-sitosterol were identified by mixed melting point determination with an authentic sample while arnebin-7, mp 94–95° (lit. mp 95°), alkannin isovalerate, mp 98° (lit. mp 94–95°), and alkannin acetate, mp 105–106° (lit. mp 104–105°), were identified by comparing IR, NMR and mass spectral data [8–10].

The major pigment of the roots is alkannin acetate. The presence of shikalkin [(±)-alkannin] reported earlier, is perhaps due to the acid/alkaline conditions employed during the course of isolation.

EXPERIMENTAL

Mps are uncorr.

Extraction and isolation. Air dried roots (3.25 kg) were extracted with hexane at room temp. The solvent was removed at red. pres. at a temp. below 50°. The dark viscous residue was chromatographed on Si gel. The column was eluted successively with hexane and hexane–C_6H_6 mixtures of increasing polarity and finally with C_6H_6. The hexane fraction was a mixture of three compounds, arnebin-7, alkannin isovalerate and alkannin acetate, which were isolated by repeated CC. A mixed solvent (3:1) fraction gave a further quantity of alkannin acetate. Subsequent elution with hexane–C_6H_6 (1:1) afforded alkannin and a mixture of alkannin and β-sitosterol which were separated by repeated CC. Elution with C_6H_6 afforded alkannin β-hydroxyisovalerate.

Alkannin β-hydroxyisovalerate (**1**). Recrystallized from hexane. UV and ^1H NMR (100 MHz, $CDCl_3$, TMS as int. standard): see text; IR ν_{max}^{Nujol} cm^{-1}: 3480 (OH), 1705 (CO, ester), 1606, 1450, 780; MS, *m/z* (rel. int.): 388 $[M]^+$ (3.3), 330 (0.55), 288 (2.5), 272 (4.4), 271 (4.7), 270 (63.3), 255 (20.3), 229 (17.5), 227 (4.4), 220 (30), 219 (100), 191 (5.5), 190 (4.8), 101 (4.4), 59 (51.6).

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