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A DAMASCONE DERIVATIVE FROM *NICOTIANA TABACUM*

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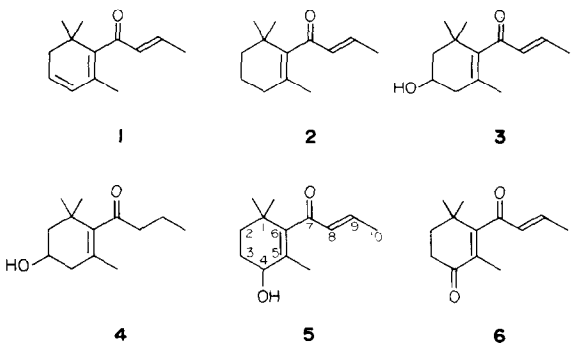
Key Word Index—*Nicotiana tabacum*; Solanaceae; Virginia tobacco; norcarotenoids; damascones.

Abstract—A new member of the damascone series, 4-hydroxy-β-damascone, was identified in the steam distillable oil from Virginia tobacco.

Chemical studies on tobacco leaf have revealed numerous volatile compounds which appear to be derived from carotenoids by oxidative degradation (see ref. [1]). Many of these are considered to contribute to tobacco aroma and possibly smoking quality and in this context damascenone (**1**) and β-damascone (**2**) are important. The unique odour properties of these compounds also ensure their popularity as constituents in fruit-type fragrance and flavour formulations.



Natural existence of **1** was first reported in 1970 when it was identified as a constituent of Bulgarian rose oil [2] and subsequently both compounds were identified in Burley tobacco [3]. More recently these compounds have been found in a wide variety of other natural products. The first report of naturally occurring hydroxyl derivatives of **2** came in 1972. Both 3-hydroxy-β-damascone* (**3**) and 3-hydroxy-8,9-dihydro-β-damascone (**4**) were identified in cigar tobacco [4] and **3** has since been found in Virginia tobacco [5, 6]. Biogenesis of these compounds is explained in terms of oxidative degradation of known C-3 oxygenated carotenoids.

We have found that a subfraction of steam distillable oil from Virginia tobacco, consisting mainly of alcohols and lactones, contained **4** and two isomers (presumably *cis* and *trans*) of **3**. A minor component of this subfraction was shown to be identical with the major component of a mixture derived by 'ageing' pure β-damascone in the presence of air. It was subsequently identified as 4-hydroxy-β-damascone (**5**), a compound which hitherto has not been reported as a constituent of any natural material.

The carbon skeleton of **5** was established by chemical oxidation to a known compound (**6**) which was also a component of the 'aged' β-damascone mixture and which was characterized using existing physicochemical data [7]. Dehydration of **5** gave **1**. Accurate mass measurement of the molecular ion gave the molecular formula as $C_{13}H_{20}O_2$. The IR spectrum indicated hydroxyl and carbonyl groups, the position of the latter absorption being typical of a conjugated unsaturated ketone. Other fragments of the structure suggested by the IR spectrum were a gem-dimethyl group and a conjugated *trans*-disubstituted olefin. The ^{1}H NMR spectrum showed a typical ABX_3-type coupling with chemical shifts and coupling constants consistent with a *trans*-crotonyl fragment. This was also strongly indicated by the mass spectrum with ions at *m/z* 69, 41 and 139 $[M-69]^+$. The absence of any further olefinic protons fixed the position of the second double bond at C-5. The position of the hydroxyl group was indicated by the multiplicity of the NMR absorption of the methine proton and confirmed by oxidation of **5** to **6**.

EXPERIMENTAL

Material. The tobacco used was a typical U.K. blend of flue-cured Virginia tobaccos.

Analytical conditions. HPLC (LDC instrumentation): conditions (A) operating pressure 10 kg/cm^2, hexane–Et_2O (17:3) isocratic (100 ml) then 1% per min to 100% Et_2O, flow rate 4.75 ml/min. Column 35 × 2.5 cm i.d., packing 40–63 μm silica, RI detector. Sample size 1 ml via loop injection. Conditions (B) as above except hexane–Et_2O (7:3) increasing 1%/min to 100% Et_2O. GC: conditions (A) 15 m WCOT CAR 20M on 0.3 mm i.d. fused silica, inj. split ratio 15:1, 1 μl samples, FID detector, 160° isothermal. Conditions (B) (also for GC/MS) as above except 70–180° at 2°/min. Conditions (C) 50 m column, as above except inj. split ratio 43:1, temp. program 70° (12 min) then to 220° at 2°/min.

Fractionation of the tobacco steam distillate. $CHCl_3$ extracts of a tobacco steam distillate, obtained by standard procedures, were dried and evaporated. The residual oil was repeatedly fractionated on Si gel (Merck, type 60, mesh 70–230) columns developed in mixtures of Et_2O and Me_2CO. A subfraction containing compounds **3**, **4** and **5** eluted when the concn of Me_2CO was *ca* 5%.

*The numbering system of carotenoid nomenclature is used here.

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 re-investigate 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.