Phytochemistry, Vol 27, No 4, pp 1191–1193, 1988
Printed in Great Britain

0031 9422/88 $3 00+0 00

DI-*O*-ACYL DERIVATIVES OF SHIKIMIC ACID FROM *SENECIO NEBRODENSIS*

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(*Revised received* 10 *August* 1987)

Key Word Index—*Senecio nebrodensis*, Compositae, sesquiterpenes, flavones, shikimic acid derivatives

Abstract—Six sesquiterpene hydrocarbons and two flavones of well known structures, together with an unresolvable mixture of tri-*O*-acyl derivatives of shikimic acid and three new di-*O*-acyl derivatives of shikimic acid, have been isolated from the aerial parts of *Senecio nebrodensis*. The structures of the new compounds were established on the basis of their spectral data and by chemical methods.

INTRODUCTION

Senecio nebrodensis L. is a perennial plant of the Spanish mountains, where it grows in nitrified green sands. It has been reported to contain large quantities of 3-ethyl-*cis*-crotonic acid together with several sesquiterpenoids (hydrocarbons and eremophilane derivatives) [1]. In the present paper, we describe the structural determination of new shikimic acid derivatives obtained from the hexane extract of the aerial parts of *S. nebrodensis*.

RESULTS AND DISCUSSION

β-Bisabolene, β-farnesene, α- and β-selinene, seline-4,11-diene and isocomene were isolated from the least polar fraction while two flavones, 3,7,4′-trimethoxy-5-hydroxyflavone and 3,7,3′,4′-tetramethoxy-5-hydroxyflavone, were obtained from the fraction of medium polarity. These compounds were identified either by comparison with authentic samples or by comparison of their physical and spectroscopic properties with those reported in the literature [2–7] for such substances. After esterification with diazomethane of the most polar fraction, several shikimic acid derivatives, as a complex mixture of tri-*O*-acyl derivatives (**1**) and three new di-*O*-acyl derivatives (**2**–**4**), were isolated

The mixture **1** showed in its IR spectrum ester carbonyl group (1748 and 1727 cm^{-1}) as well as carbon–carbon double bond absorptions (1650 cm^{-1}). Its ^{1}H NMR spectrum showed signals corresponding to a methylene (δ2.65, *m*) and a methoxyl group (δ3.78, *s*), geminal hydrogens to an *O*-acyl group (δ5.33 and 5.65, *m*) and a vinylic hydrogen (δ6 68, *m*), all of which were assignable to a methyl shikimate skeleton. Methyl signals appearing at δ0.97 (*t*, $J = 8$ Hz), 1 07 (*t*, $J = 8$ Hz), 1 90 (*br s*) and 2.16 (*br s*), as well as at δ1 26 (*m*, $-CH_2-$) and 5 68 (*m*, HC=C) were assigned to three different acids. 3-ethyl-*cis*-crotonic, senecioic and caproic acid, in decreasing order of abundance. Saponification of **1** confirmed the above, since the afore mentioned acids together with shikimic acid, all as 4-phenylphenacylic esters, were isolated.

The second substance, in increasing order of polarity, had the molecular formula $C_{20}H_{28}O_7$ (*m*/*z* 380[M]$^+$) The IR spectrum contained absorption bands for hydroxyl (3497 cm^{-1}) and ester carbonyl groups as well as trisubstituted carbon–carbon double bonds similar to those of **1** Its ^{1}H NMR spectrum (see Table 1) also showed characteristic signals of a methyl shikimate derivative The hydroxyl group was localized at C-4 by means of double resonance experiments Thus, the signal at δ4 05, which appeared as a double doublet ($J = 7.2$ and 4 0 Hz), was converted into a doublet by irradiation of H-3 and H-5 The remaining signals were in accordance with the presence of two *O*-acyl groups at C-3 and C-5 both with the structure of 3-ethyl-*cis*-crotonate In the mass spectrum, the presence of a intense peak at *m*/*z* 266 (17.1%) was remarkable, and was due to the loss of 3-ethylcrotonic acid This is in agreement with the data previously reported in the literature [8, 9], where an easy elimination of groups in allylic positions is described. The above data together with the ^{13}C NMR data alloweded us to assign structure **2** to this compound In addition, acetylation of this substance in the usual manner afforded a mono-*O*-acetyl derivative (**2a**), spectroscopic data of which also supported the proposed structure

The other two new compounds (**3** and **4**) were shown to be isomers of **2** Thus, the mass spectra of both compounds contained a peak at *m*/*z* 380 (M$^+$, $C_{20}H_{28}O_7$), and their IR spectra exhibited absorption bands very similar to those of **2**. That the free hydroxyl group in **3** was at C-3 was established by ^{1}H NMR Thus, H-3 and H-4 undergo an upfield and downfield shift, respectively, in relation with their chemical shift in **2**, reflecting the change of the position of the *O*-acyl group. Double resonance experiments and the relative intensity (6.2%) of the fragment ion at *m*/*z* 266 also confirmed the above statement On the other hand, the free hydroxyl group in **4** was at C-5. This was demonstrated by the fact that the proton under the above hydroxyl group was coupled with the methylene group, as was shown by double resonance experiments.

On acetylation, **3** and **4** afforded the corresponding 3-*O*-(**3a**) and 5-*O*-acetyl derivative (**4a**), the spectroscopic and analytical data of which were in accordance with those expected for such compounds. The upfield shift (3.3 ppm) of the C-6 signal in **4a** with respect to that in **4**, is probably due to a γ-effect and is characteristic.

Table 1. ^{1}H NMR spectral data of compounds **2–4** and **2a–4a** (80 MHz, CDCl$_3$, TMS as int. standard)

H	**2**	**2a**	**3**	**3a**	**4**	**4a**
2	6.81 *m*	6.81 *m*	6.81 *m*	6.77 *m*	6.81 *m*	6.80 *m*
3	5.69 *m*	5.80 *m*	4.62 *m*	5.77 *m*	5.81 *m*	5.77 *m*
4	4.05 *dd* (7.2, 4.0)*	5.35 *m*	5.29 *m*	5.35 *m*	5.08 *dd* (8.9, 4.3)	5.32 *m*
5	5.20 *m*	5.35 *m*	5.29 *m*	5.35 *m*	4.25 *m*	5.32 *m*
6	2.65 *m*	2.72 *m*	2.45–2.87 *m*	2.68 *m*	2.80–3.15 *m*	2.68 *m*
8	3.75 *s*	3.77 *s*	3.76 *s*	3.77 *s*	3.78 *s*	3.75 *s*
2′	5.69 *m*	5.65 *m*	5.63 *m*	5.62 *m*	5.63 *m*	5.62 *m*
4′	2.19 *q* (8.0)	2.30 *q* (7.5)	2.18 *q* (8.0)	2.20 *q* (7.5)	2.20 *q* (8.0)	2.20 *q* (7.5)
5′	1.08 *t* (8.0)	1.08 *t* (7.5)	1.08 *t* (8.0)	1.06 *t* (7.5)	1.08 *t* (8.0)	1.06 *t* (7.5)
6′	2.16 *d* (1.6)	2.16 *d* (1.5)	2.17 *d* (1.5)	2.14 *d* (1.5)	2.15 *d* (1.5)	2.13 *d* (1.5)
OAc	-	2.02 *s*	—	2.06 *s*	--	2.05 *s*

*Coupling constants (*J* in Hz) are given in parentheses.

Table 2. ^{13}C NMR chemical shifts of compounds **2–4** and **2a–4a** (20 MHz, CDCl$_3$, TMS as int. standard)

C	**2**	**2a**	**3**	**3a**	**4**	**4a**
1	131.2	130.9	129.2	130.9	131.7	130.8
2	133.4	133.3	136.9	133.4	133.4	133.6
3*	68.0	65.1	65.4	66.2	65.4	65.3
4*	68.4	65.8	66.2	66.4	65.7	66.8
5*	68.6	68.2	69.1	66.7	71.3	66.9
6	28.4	28.6	27.8	28.2	31.6	28.3
7	166.3	165.4	166.1	163.4	165.7	165.2
8	52.1	52.0	52.1	52.1	52.1	51.9
1′	166.3	166.0	166.6	165.5	166.2	165.8
2′	113.5, 114.0	113.6	113.4, 113.8	113.6, 113.8	113.8	113.3, 113.5
3′	163.4	163.3, 163.5	163.4	163.4	163.4	163.1
4′	33.9	33.7	33.9	33.8	33.8	33.6
5′	11.9	11.8	11.9	11.8	11.9	11.7
6′	19.0, 19.1	18.8	19.0, 19.1	18.9	19.0	20.7
Me$\underline{\text{C}}$O	---	169.7		169.9	--	169.6
MeCO	-	20.6	--	20.8		20.7

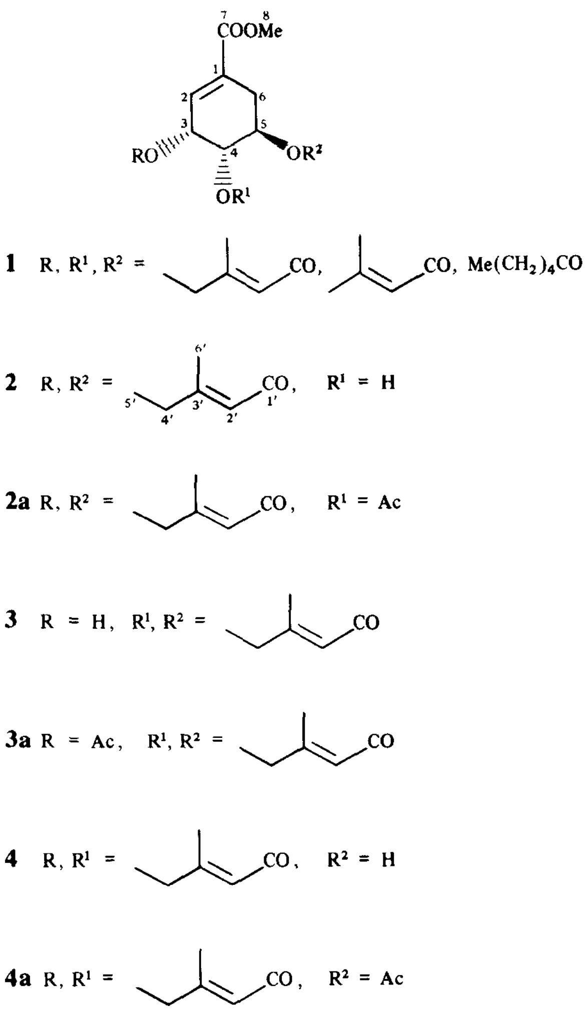
*These carbons may be interchanged.

These findings constitute a new contribution to the phytochemical significance of shikimic acid derivatives in the genus *Senecio* [8–11].

EXPERIMENTAL

Mps uncorr. IR film or KBr; ^{1}H (80 MHz) and ^{13}C NMR (20 MHz) CDCl$_3$ or DMSO-d_6, using TMS as int. standard. Carbon substitution degrees were established by DEPT pulse sequence. MS 70 eV. Analytical TLC silica gel G (Merck 7331), CC silica gel 60 (Merck 7734), flash CC silica gel (Merck 7729).

The plant was collected in June 1986 in Sierra Nevada, 2500 m altitude (Granada, Spain) and was identified by Professor F. Valle (Department of Botany, University of Granada). A voucher specimen is deposited in the Herbarium of the Faculty of Sciences of the University of Granada (GDAC, n.r.). The air-dried aerial parts (419 g) were extracted with hexane (6 l) in a Soxhlet apparatus to afford an extract (35 g, 8.35% from dried plant), which was dissolved in hot MeOH, cooled to 0°, filtered, and the filtrate concentrated to give a residue (25 g), a part of which (15.1 g) was subjected to flash CC (150 g of silica gel) eluting with hexane–Et$_2$O mixtures (19:1, 4:1, 1:1), Et$_2$O, EtOAc and EtOAc–AcOH (9:1) successively. From the first fraction, after CC (AgNO$_3$–silica gel 1:4), *β*-bisabolene (1 mg), *α*-selinene (7 mg), *β*-farnesene (1 mg), *β*-selinene (72 mg), selina-4,11-diene (4 mg) and isocomene (2 mg) were isolated. From the second fraction, eluted with hexane–Et$_2$O (1:1), and after crystallization from MeOH, 3,7,4′-trimethoxy-5-hydroxyflavone (3 mg, mp 146–147°, lit. [6] 147–149°) and 3,7,3′,4′-tetramethoxy-5-hydroxy flavone (15 mg, mp 158–159°, lit. [7] 160–161°) were obtained. Finally, the most polar fraction, eluted with EtOAc–AcOH (9:1), was treated at −15° with CH$_2$N$_2$, and the residue chromatographed to yield **1** (4.16 g), **2** (252 mg), **3** (41 mg) and **4** (63 mg).



Mixture of methyl shikimate tri-O-acyl derivatives (**1**) Colourless oil GC/MS *m/z* (rel int) 478 (0 1), 464 (0 2), 450 (0.2), 364 (1 4), 350 (2 0), 336 (1 0), 268 (2 2), 252 (3 3), 170 (4 5), 152 (7 1), 97 (100) IR ν_{max}cm^{-1} 2966, 2940, 2879, 1748, 1650, 1439, 1252, 1222, 1139

Methyl 3,5-di-O-(3-methyl-(E)*-2-pentenoyl)shikimate* (**2**) Colourless oil GC/MS *m/z* (rel int): 380 $[M]^+$ (0 7), 362 $[M-H_2O]^+$ (1 3), 349 $[M-MeO]^+$ (1 5), 267 (4 6), 266 (17 1), 170 (4 9), 152 (3 7), 97 (100) IR ν_{max}cm^{-1}. 3497, 3020, 2970, 2931, 2881, 1727, 1649, 1439, 1254, 1221, 1143, 1102 Acetate (**2a**) oil IR ν_{max}cm^{-1} 2970, 2940, 2881, 1749, 1746, 1740, 1725, 1648, 1437, 1369, 1235, 1221, 1141, 1102, 1074

Methyl 4,5-di-O-(3-methyl-(E)*-2-pentenoyl)shikimate* (**3**) Colourless oil GC/MS *m/z* (rel int) 380 $[M]^+$ (0 1), 365 $[M-Me]^+$ (0 1), 362 $[M-H_2O]^+$ (0 2), 349 $[M-MeO]^+$ (0 1), 267 (3 3), 266 (6 2), 170 (4 4), 152 (2 3), 97 (100) IR ν_{max}cm^{-1}. 3485, 3020, 2969, 2939, 2881, 1727, 1649, 1439, 1250, 1220, 1143, 1101 Acetate (**3a**) oil IR ν_{max}cm^{-1} 2964, 2937, 1742, 1722, 1647, 1437, 1370, 1228, 1136, 1100, 1040

Methyl 3,4-di-O-(3-methyl-(E)*-2-pentenoyl)shikimate* (**4**) Colourless oil GC/MS *m/z* (rel int) 380 $[M]^+$ (0 5), 362 $[M-H_2O]^+$ (0 7), 349 $[M-MeO]^+$ (0.1), 267 (6 4), 266 (14 5), 170 (28 8), 152 (13 9), 97 (100) IR ν_{max}cm^{-1} 3497, 3202, 2960, 2938, 2880, 1727, 1649, 1439, 1251, 1219, 1140, 1101, Acetate (**4a**): oil IR ν_{max}cm^{-1} 2968, 2938, 2882, 1760, 1746, 1726, 1708, 1644, 1435, 1369, 1218, 1144, 1093, 1045

*Isolation and identification of shikimic, 3-ethyl-*cis*-crotonic, senecioic and caproic acid* The mixture **1** (2 g) was treated with 2 M KOH in MeOH at room temp for 24 hr After evaporation of the solvent the residue was suspended in H_2O (15 ml), then acidified to pH 2 with 2 M HCl, and extracted with Et_2O The aq phase was adjusted to pH 6 with aq NaOH, concentrated and the residue extracted with hot EtOH Concentration of the extract yield a colourless residue (0 6 g) The residue was suspended in EtOH (60 ml), the pH adjusted to 7 2, and 4-phenylphenacyl bromide (1 6 g) added, and the mixture heated under reflux for 30 min The reaction mixture was concentrated and the residue treated with H_2O and extracted with EtOAc Concentration of the extract afforded colourless crystalline 4-phenylphenacyl shikimate (0 27 g) Mp 161–162° (hexane) GC-MS *m/z* (rel int) 368 $[M]^+$ (3 2), 350 $[M-H_2O]^+$ (1 1), 332 (1 0), 309 (2 9), 291 (2 8), 278 (2 5), 213 (10 0), 181 (100), 153 (11 2), 152 (19 8) IR ν_{max}^{KBr} cm^{-1} 3342, 3034, 2933, 2916, 1737, 1696, 1660, 1607, 1418, 1222, 1107, 1068 ^{1}H NMR (DMSO-d_6) δ 1 95–2 60 (2H, *m*, H-6), 3 67 (1H, *m*, H-5), 3 94 (1H, *m*, H-4), 4.33 (1H, *m*, H-3), 4 68 (1H, *d*, $J = 4$ Hz, C_5–OH), 4 90 (2H, *m*, C_3–OH and C_4–OH), 5 59 (2H, *s*, $-CH_2-$), 6.79 (1H, *m*, H-2), 7 40–8 20 (9 Ar–H, *m*) $[\alpha]_{589} - 20\ 0°$ (MeOH; *c* 0 99)

A soln of **1** (1 9 g) in Et_2O (50 ml) was shaken with 4% aq NaOH (3 × 50 ml) The combined aq extracts were acidified with HCl to pH 2, then extracted with Et_2O The organic phase was concd to yield a mixture (0.9 g) of 3-ethyl-*cis*-crotonic, senecioic and caproic acids, which were identified after separation by CC as their corresponding 4-phenylphenacyl esters

Acknowledgement—We thank Professor F Valle (Department of Botany, Faculty of Sciences, University of Granada) for identification of the plant material

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