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New stilbene carboxylic acid from *Convolvulus hystrix*

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Convolvulus hystrix Vahl. (family Convolvulaceae) is a common wild plant growing in the kingdom of Saudi Arabia. It is used in traditional medicine. Our study resulted in the isolation and structural elucidation of ten natural products from which a stilbene carboxylic acid was isolated for the first time as a new natural product. The isolated products are mainly shikimates in addition to two sterols; β-sitosterol and stigmasterol, as well as one triterpenoid, oleanolic acid. The shikimate products are vanillin, vanillic acid, syringic acid, ferulic acid, isoferulic acid, isoscopoletin and the new stilbene derivative. The structures were elucidated on the basis of spectral data from 400 MHz ^{1}H NMR, MS, IR and UV. The biogenesis of the new stilbene is discussed.

1. Introduction

Convolvulus hystrix Vahl. (family Convolvulaceae) is a common wild plant growing in the kingdom of Saudi Arabia and used in traditional medicine as a purgative [1]. Only the coumain scopoletin has been isolated from *C. hystrix* [2]. Otherwise, no information about the chemical constituents of this plant has been found in the literature. Typical compounds of the genus *Convolvulus* are alkaloids [3–5] as well as flavonoids, triterpenoids, sterols, cinnamic acid derivatives, n-alkanes and n-alkanols [6–8]. In this paper, we present our results of the chemical investigation of *C. hystrix* including a new stilbene carboxylic acid, six known shikimate aromatics, β-sitosterol, stigmasterol and oleanolic acid.

2. Investigations, results and discussion

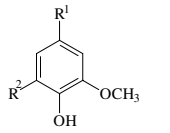
An extract of *Convolvulus hystrix* yielded a new stilbene derivative **7**, in addition to the known compounds **1**–**6**, β-sitosterol, stiginasterol and oleanolic acid. β-Sitosterol [9] and stigmasterol [9], in a mixture, were identified from their characteristic signals of H-6, H-3, together with H-22 and H-23 of stigmasterol in the ^{1}H NMR spectrum. Oleanolic acid [10] was identified from its characteristic signals of H-3, H-12 and H-18 in the ^{1}H NMR spectrum. The ^{1}H NMR spectra of compounds **1** and **3** were nearly similar. They showed three aromatic proton signals of a 1,3,4-trisubstituted benzene ring. In addition, a methoxyl group singlet was seen in each spectrum and an aldehydic proton signal at δ 9.92 in the spectrum of **1**. Thus, **1** was suggested to be vanillin [9] and **3** vanillic acid [9].

Compound **2** showed M^+ at m/z 198 corresponding to $C_9H_{10}O_5$, with a methoxyl group more than **3**. This was in accordance with the ^{1}H NMR spectrum, which showed only two singlets at δ 7.38 for two aromatic protons and δ 3.96, for two methoxyl groups. Thus, the molecule of **2** consists of two similar halves. This was consistent with syringic acid [11].

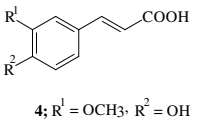
The ^{1}H NMR spectra of compounds **4** and **5** were very similar with slight differences in the chemical shifts of the signals. Both spectra showed the signals of *trans*-3,4-disubstituted cinnamic acid and only one methoxyl group singlet. That indicates the presence of ferulic and isoferulic acids. The methoxyl group singlet in the spectrum of **4** appeared more upfield (at δ 3.82) relative to that of **5** (at δ 3.94). Thus **4** was assigned as ferulic acid [12] and **5** as isoferulic acid [13].

Compound **6** showed a molecular ion peak, M^+ at m/z 192 corresponding to $C_{10}H_8O_4$, with two H atoms less than **4** or **5**. The ^{1}H-NMR spectrum showed only two aromatic proton singlets (*para* to each others) instead of three in the spectrum of **4** or **5**. This suggested δ-lactone ring closure, which was consistent with the *cis* coupling of the olefinic protons, of 9.5 Hz instead of 16 Hz in **4** or **5**. Thus, **6** is either scopoletin or isoscopoletin. The UV spectra of **6**, before and after addition of sod. acetate showed no significant shift in λ_{max} at 333 nm. Thus, according to Horowitz *et al*. [14], **6** is isoscopoletin [15].

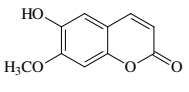
The MS of compound **7** showed M^+ at m/z 342 and a base peak at m/z 298 due to $[M\text{-}CO_2]^+$. High resolution of the base peak gave 298.1205 in accordance with the formula $C_{18}H_{18}O_4$. Thus, **7** is a carboxylic acid having the molecular formula $C_{19}H_{18}O_6$. The IR spectrum showed absorption bands at 3540 cm^{-1} (free OH), 1710 cm^{-1} (COOH), 1640 and 1615 cm^{-1} (C=C) and 1520 cm^{-1} (aromatic ring).



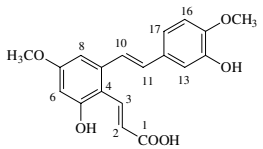
1; R^1 = CHO, R^2 = H
2; R^1 = COOH, R^2 = OCH_3
3; R^1 = COOH, R^2 = H



4; R^1 = OCH_3, R^2 = OH
5; R^1 = OH, R^2 = OCH_3



6



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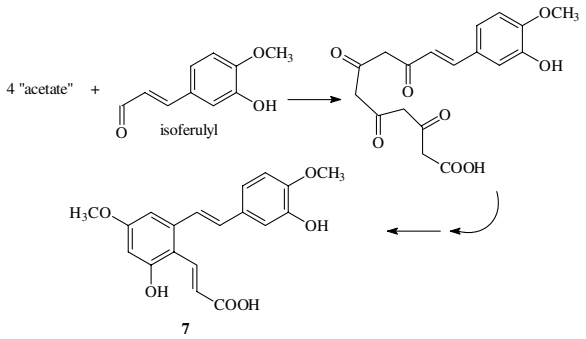
The ^{1}H NMR spectrum (Table) showed the signals of two *trans*-1,2-disubstituted double bonds. One was attributed to cinnamic acid residue [δ 7.63 *d*(16) and δ 6.29 *d*(16)] and the second was attributed to stilbene double bond [δ 7.20 *d*(16.5) and δ 7.10 *d*(l6.5)]. The substitution pattern of the two phenyl rings was decided from the proton spectrum. One ring was 1,2,4-trisubstituted [δ 7.06 *d*(2), δ 7.02 *dd*(8.2) and δ 6.86 *d*(8)] and the other ring was 1,2,3,5-tetrasubstituted [δ 7.33 *d*(2) and δ 6.92 *d*(2)]. Hence the two signals of the latter ring shifted downfield and the value of shift is small, indicating the ethenyl carboxylic acid side chain in that ring to be located in the *meta* position to both protons.

Biogenetically, this C_3-side chain, together with the "acetate" ring must have originated from four "acetate" units, instead of three in the case of known stilbenes [16]

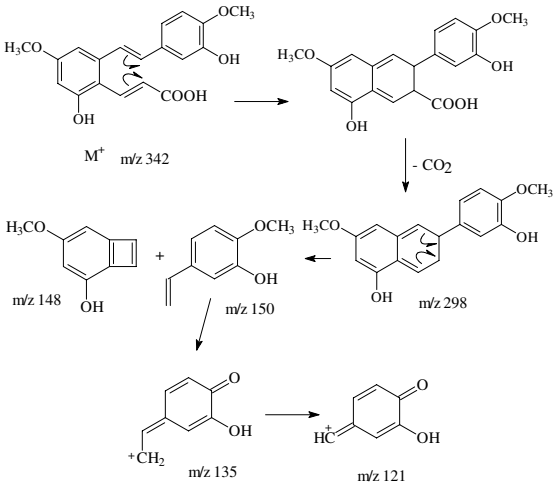
Table: ^{1}H NMR data of stilbene 7 (400 MHz, $CDCl_3$)

H-atom	δ-value	multiplicity (J, Hz)
H-2	6.295	d (16)
H-3	7.63	d (16)
H-6	6.92	d (2)
H-8	7.33	d (2)
H-10	7.10	d (16.5)
H-11	7.20	d (16.5)
H-13	7.06	d (2)
H-16	6.86	d (8)
H-17	7.02	dd (8,2)
C-7-OCH_3	3.93	s
C-14-OCH_3	3.92	s

Scheme 1



Scheme 2



and we suggest the biogenetic conversion of isoferulic acid **5** to **7** (Scheme 1).

The ^{1}H NMR spectrum showed two methoxyl group singlets and the MS fragmentation (Scheme 2) established one methoxyl group in each ring.

The down-field shift of the two methoxyl group signals (δ 3.93 *s* and δ 3.92 *s*) was attributed to the mesomeric deshielding of the carboxylic group. Thus, the methoxyl group in the "shikimate" ring must be in the *para* position. According to previous work [17], the biosynthetic methylation of the 7-hydroxyl group seems to be easier and more frequent, while at position 5, methylation does not take place unless all positions have been methylated. Consequently, the methoxyl group in the "acetate" ring was located at position 7, and **7** was identified as 3-(3,3′-dihydroxy-5,4′-dimethoxy-2-E-stilbenyl)-E-propenoic acid. The structures of the known compounds were confirmed by comparing the ^{1}H NMR spectra with authentic ones.

3. Experimental

3.1. Plant material

Convolvulus hystrix Vahl. (family Convolvulaceae) was collected and identified by Prof. Dr. L. Boulus, Professor of Plant Taxonomy, National Research Center, Dokki, Cairo, Egypt, from the Campus of King Abdulaziz University, Jeddah, Saudi Arabia.

3.2. Processing of the plant material

The dried plant material (650 g, whole plant,) was soaked in a solvent mixture of petroleum ether/ether/methanol (1 : 1 : 1) at room temperature for about 24 h. The extract (10.2 g) was defatted using cold methanol.

3.3. Separation of the compounds

The defatted extract ((5 g) was chromatographed (4 g of the extract) using a silica gel CC (270 g) eluted with pet ether containing increasing amounts of ether, then with ether containing small amounts of methanol.

Fraction I (350 mg, eluted with pet ether/ether 2 : 1) was reseparated by preparative TLC (silica gel, methyl t-butyl ether (MTBE)/pet. ether 1 : 3) into three compounds; **5** (60 mg, R_f 0.39), **2** (20 mg, R_f 0.27) and **7** (40 mg, R_f 0.15).

Fraction II (80 mg, eluted with pet ether/ether 1 : 1) yielded a mixture of β-sitosterol and stigmasterol (m.p. 120–122 °C).

Fraction III (300 mg, eluted with ether) gave by preparative TLC (silica gel, MTBE/pet. ether 1 : 1) two compounds; **3** (60 mg, R_f 0.54) and **6** (60 mg, R_f 0.25).

Fraction IV (20 mg, eluted after III by ether) yielded oleanolic acid which was purified by TLC (silica gel, MTBE/pet. ether 5 : 1) at R_f 0.59.

Another portion of the extract (1 g) was partitioned between 10% KOH solution and chloroform. The aqueous layer was acidified with 10% HCl solution, then extracted with chloroform. The $CHCl_3$-extract (340 mg) gave by preparative TLC (silica gel, MTBE/pet. ether 3 : 1) two compounds; **1** (42 mg, R_f 0.74) and **4** (50 mg, R_f 0.35).

*3.4. 3-(3,3′-Dihydroxy-5,4′-dimethoxy-2-E-stilbenyl)-E-propenoic acid (**7**)*

Yellowish gummy material, IR ν_{max}, cm^{-1}: 3540 (OH), 1710 (COOH), 1640, 1615 (C=C), 1520 (aromatic ring); ^{1}H NMR, *cf.* Table; MS, m/z (rel. int.): 342 $[M]^+$ (8%), 298.1205 $[M\text{-}C0_2]^+$ (calc. for $C_{18}H_{18}0_4$: 298.3417) (100%), 150 (30%), 149 (28%), 148 (35%), 137 (27%), 135 (60%), 121 (19%).

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