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Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713454007>

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To cite this Article Ahmed, Bahar , Al-Howiriny, Tawfeq A. , Mossa, Jaber S. and Al-Said, Mansour S.(2004) 'Three new and two rare furanoeremophilanes from *senecio asirensis*', Journal of Asian Natural Products Research, 6: 3, 167 – 175

To link to this Article: DOI: 10.1080/1028602032000169596

URL: <http://dx.doi.org/10.1080/1028602032000169596>

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THREE NEW AND TWO RARE FURANOEREMOPHILANES FROM *SENECIO ASIRENSIS*

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(Received 8 July 2003; Revised 24 September 2003; In final form 21 October 2003)

Three new furanoeremophilanes have been obtained from the aerial parts of *Senecio asirensis* (N. O. Asteraceae), and characterized as 6-hydroxymethyl-9-methoxyl-4,11-dimethylnaphtho[2,3-*b*]furan, designated asirensane-a (**1**), 6-hydroxyl-1,2-dimethoxyl-4,6,11-trimethyl-6-hydronaphtho[2,3-*a*]furan-7-one, named asirensane-b (**2**), and (6,12-dihydroxyl-9-methoxyl-4-methyl-11-acetyl-3,4-dihydronaphtho[2,3-*b*]furan-3-yl)methyl (2'*Z*)-2'-methyl-but-2'-enoate, designated asirensane-c (**3**). In addition, two rare furanoeremophilanes have also been isolated and characterized from this source, namely 9-methoxyl-4,11-dimethylnaphtho[2,3-*b*]furan, named 14-nordehydrocalohastine (**4**), and 4,11-dimethylnaphtho[2,3-*b*]furan-6,9-dione, designated as maturinone (**5**). Their structures have been elucidated on the basis of spectral analysis. The alcoholic extract was also tested for anti-inflammatory activity, which decreased edema by 22% at a dose of 500 mg kg⁻¹ after 3 h with respect to the control group treated only with carrageenan, while the standard drug phenylbutazone showed a 50% decrease at a dose of 100 mg kg⁻¹, indicating that the extract has moderate anti-inflammatory activity.

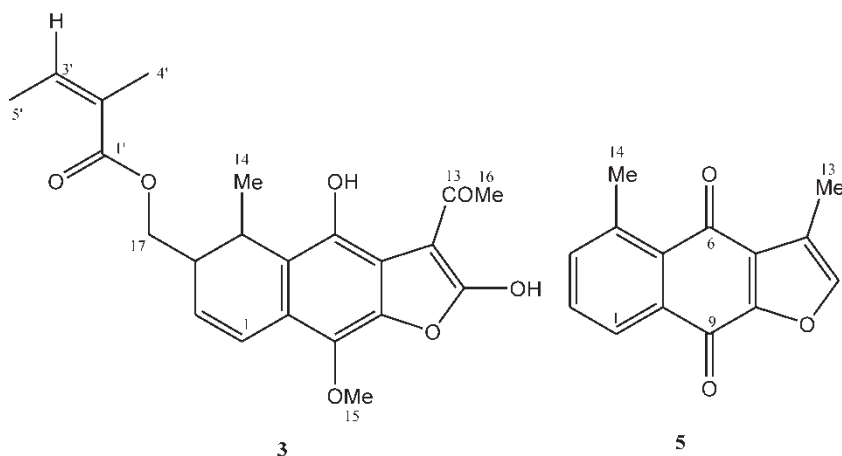
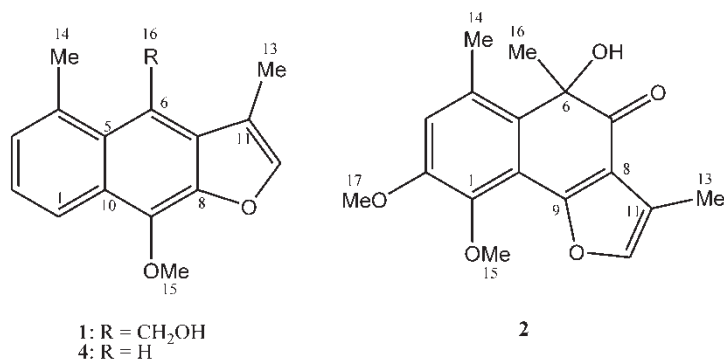
Keywords: *Senecio asirensis*; Furanoeremophilane; Asirensane-a, b, c; 14-Nordehydrocalohastine; Maturinone; Anti-inflammatory activity

INTRODUCTION

The genus *Senecio* (Family: Asteraceae) is widely distributed throughout the world and is a source of pyrrolizidine alkaloids, eremophilanolides and furanoeremophilanes [1–3]. Plants containing pyrrolizidine alkaloids exhibit hepatotoxic activity and a broad range of other pharmacological actions such as mutagenic, cytotoxic, genotoxic, antifeedent and antifungal activity, and are considered a health hazard for both humans and livestock [4,5]. The plant *Senecio asirensis* Boulos & JRI Wood commonly known as Baidha and Hashma in Arabic is distributed in Saudi Arabia and Yemen. A literature survey revealed no systematic phytochemical and pharmacological work on this plant. We have isolated three new furanoeremophilanes from the aerial parts of the plant, which have been characterized as 6-hydroxymethyl-9-methoxyl-4,11-dimethylnaphtho[2,3-*b*]furan, designated

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as asirensane-a (**1**), 6-hydroxyl-1,2-dimethoxyl-4,6,11-trimethyl-6-hydronaphtho[2,3-*a*]furan-7-one, named asirensane-b (**2**), and (6,12-dihydroxyl-9-methoxyl-4-methyl-11-acetyl-3,4-dihydronaphtho[2,3-*b*]furan-3-yl)methyl (2'*Z*)-2'-methylbut-2'-enoate, designated asirensane-c (**3**). Two rare furanoeremophilanes were also isolated and characterized from this source, namely 9-methoxyl-4,11-dimethylnaphtho[2,3-*b*]furan, named 14-nordehydrocalohastine (**4**, earlier reported also from *Senecio linifolius* [6]), and 4,11-dimethylnaphtho[2,3-*b*]furan-6,9-dione designated maturinone (**5**, previously isolated also from *Cacalia decomposita* [7]). The genus *Senecio* is mainly reported to possess eremophilanolides/eudesmanolides that contain an unsaturated cyclic system, and an α,β -lactone in the furan ring [8], whereas the compounds obtained from *Senecio asirensis* possess a saturated cyclic ring system without an α,β -lactone ring in the furan ring, which are less common among reported eremophilanolides.



RESULTS AND DISCUSSION

Compound **1**, named asirensane-a, obtained as light yellow needles, has a molecular formula of C₁₆H₁₆O₃, as established on the basis of HR-MS, elemental analysis, ¹³C NMR and DEPT spectra. The IR spectrum indicates hydroxyl (3450 cm⁻¹), methoxyl (1247, 1220 cm⁻¹), furan ring (811, 779 cm⁻¹) and aromatic double bonds (1631, 1602, 1504, 685 cm⁻¹). The ¹³C NMR and DEPT spectra [9] showed 16 carbon atoms for the molecule, consisting of two methyls, one methoxyl, one methylene, four aromatic methines, and eight quaternary carbon atoms

(in total $C_{16}H_{16}$). The sequential assignments of protons and carbon atoms were made with the help of $^1H-^1H$ COSY and HMQC experiments, starting with the easily distinguishable aromatic protons at δ_H 7.50 (δ_C 144.4) (assignable at position 12) and 8.28 (120.6) (attributable to position 2), and further correlated with the HMBC spectrum. The proton at δ_H 8.28 (dd, $J = 6.0, 8.5$; δ_C 120.6) correlates with protons at 7.35 (dd, $J = 1.5, 6.0$; 123.5, H-1) and 7.33 (dd, $J = 1.5, 8.5$; 129.1, H-3) in the $^1H-^1H$ COSY spectrum, indicating the consecutive location of these protons at positions 2, 1 and 3 respectively of ring A, which were substantiated by long-range couplings in the HMBC spectrum. H-1 shows long-range coupling in the HMBC spectrum with C-2 and C-10 (131.1). The methoxyl group at δ_H 4.31 (δ_C 61.1) also displays a long-range correlation with C-9, indicating it is at position 9 of ring B. The proton at position 3 exhibits long-range correlations with C-4 (126.5) and Me-14 (24.0), whereas Me-14 correlates with C-4, C-5 (134.4) and C-2, indicating its attachment at position 4. The other methyl group at δ_H 2.55 (d, $J = 1.0$) shows long-range correlations with C-11 (116.2), C-7 (133.1) and C-12 (144.4); whereas the proton at δ_H 7.50 (d, $J = 1.0$) couples with C-11, C-7 and C-8 (142.7), suggesting the methyl group is at position 11 and the proton at position 12 of ring C. In addition, the C-12 (144.4) and C-8 (142.7) appear downfield, which could suggest that the oxygen atom of the ether linkage is between C-12 and C-8 of the furan ring C. The broad signal at δ_H 5.08 (δ_C 66.2), due to a methylene group, exhibits a long-range correlation in the HMBC spectrum with C-6 (120.4), C-5 (134.4), and C-7 (133.1), indicating that it is attached at position 6. Other correlations in the HMBC spectrum accord with the proposed structure (Fig. 1, Table I).

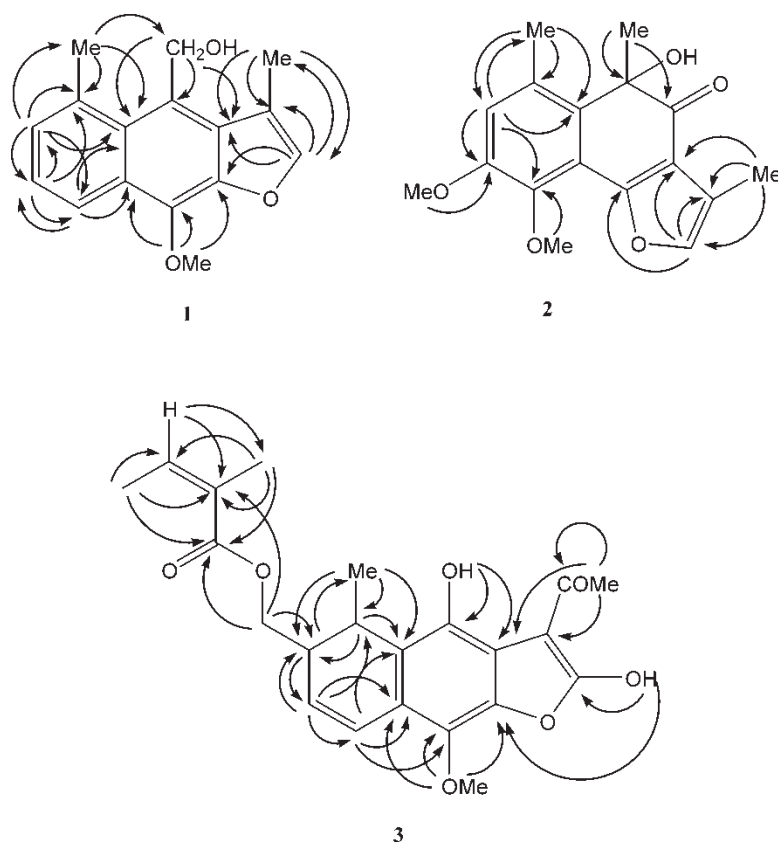


FIGURE 1 Significant HMBC correlations of 1–3.

Thus on the basis of above spectral analysis the structure of compound **1** was established as 6-hydroxymethyl-9-methoxyl-4,11-dimethylnaphtho[2,3-*b*]furan, and has been designated as asirensane-a.

Compound **2**, named asirensane-b, also obtained as light yellow needles, has a molecular formula $C_{17}H_{18}O_5$, as established on the basis of HR-MS, elemental analysis, ^{13}C NMR and DEPT spectra. The IR spectrum indicates hydroxyl (3450 cm^{-1}), methoxyl (1245 , 1222 cm^{-1}), and ketonic groups (1745 cm^{-1}), and a furan ring (840 , 798 , 745 cm^{-1}) and aromatic double bonds (1630 , 1600 , 1505 , 670 cm^{-1}). ^{13}C NMR and DEPT spectra [9] show 17 carbon atoms for the molecule, consisting of three methyls, two methoxyls, two aromatic methines, one ketonic, eight aromatic and one aliphatic quaternary carbon atoms (in total $C_{17}H_{18}$). Sequential assignments of protons and carbon atoms were made with the help of 1H - 1H COSY and HMQC experiments, starting with the easily distinguishable aromatic protons at δ_H 7.28 (δ_C 145.3) (assignable to position 12) and 6.69 (120.2) (attributable at position 3), and further correlated with the HMBC spectrum. The proton at δ_H 6.69 (δ_C 120.2), assignable at position 3 of the ring A, shows long-range correlations in HMBC spectrum with Me-14 at 2.66 (22.4), C-5 (138.3), C-2 (152.3) and C-1 (147.9); whereas Me-14 displays long-range coupling with C-4 (125.), C-3 (120.2) and C-5 (138.3), suggesting that Me-14 is at C-4. The methoxyl-17 (δ_H 3.70, δ_C 55.6) shows correlation with C-2, allowing it to be placed at position 2; and methoxyl-15 (δ_H 3.73, δ_C 61.2) correlates with C-1, indicating that it is linked at position 1. NOEs observed between H-3 and OMe-17, along with the absence of any NOE between H-3 and OMe-15, indicate the attachment of these methoxyl groups at C-2 and C-1, respectively. The Me-16 at δ_H 1.71 (27.7) displays long-range correlations with C-6 (70.9), C-5, C-7 (172.9, ketonic group) and C-8 (140.8), suggesting the placement of Me-16 at C-6, and the ketonic group at position 7 of ring B. Similarly, the other proton at δ_H 7.28 (δ_C 145.3), attributable to position 12, shows long-range correlation in the HMBC spectrum with C-11 (δ_C 120.6), C-8 and C-9 (144.8); whereas Me-13 correlates with C-11, C-8 and C-12, indicating the placement of Me-13 at position 11, and the proton at position 12 of the furan ring C. In addition, C-12 (143.3) and C-9 (144.8) appear downfield, indicating that the oxygen atom of the ether linkage is between C-12 and C-9 of the furan ring C. The 1H NMR spectrum has a one-proton singlet at δ_H 3.09 due to a hydroxyl group, which shows long-range correlation with C-6 (δ_C 70.9), indicating its attachment at proton 6 of ring B. Other correlations in the HMBC spectrum accord with the proposed structure (Fig. 1, Table I).

Thus, on the basis of above spectral analysis, the structure of compound **2** was established as 6-hydroxyl-1,2-dimethoxyl-4,6,11-trimethyl-6-hydronaphtho[2,3-*a*]furan-7-one, and has been designated as asirensane-b.

Compound **3**, named asirensane-c, obtained as light yellow semi-solid, has a molecular formula $C_{22}H_{24}O_7$, as established on the basis of HR-MS, elemental analysis, ^{13}C NMR and DEPT spectra. The IR spectrum indicates hydroxyl (3445 cm^{-1}), methoxyl (1245 , 1220 cm^{-1}), and ketonic groups (1745 , 1750 cm^{-1}), and a furan ring (838 , 798 , 740 cm^{-1}), and aromatic double bonds (1632 , 1605 , 1505 , 683 cm^{-1}). ^{13}C NMR and DEPT spectra [9] show 22 carbon atoms for the molecule, consisting of four methyls, one methoxyl, one methylene, five methines, two ketonic, nine aromatic quaternary carbon atoms (in total $C_{22}H_{24}$). Sequential assignments of protons and carbon atoms were made with the aid of 1H - 1H COSY and HMQC experiments, starting with the easily distinguishable protons at δ_H 4.05 (δ_C 68.3) (assignable to position 3) and 3.35 (36.6) (attributable to position 4), and further correlated with the HMBC spectrum. The protons at δ_H 6.89 (d, $J = 9.5$; δ_C 122.7) and 6.22 (dd, $J = 9.5$, 6.5 ; 128.7) correlate with each other in the 1H - 1H COSY spectrum and can be placed at positions 1 and 2, respectively. H-2 correlates with the proton at 4.05 (dd, $J = 4.0$, 6.5 ; δ_C 68.3), attributable to position 3; H-3 correlates with H-4 at δ_H 3.35 (dd, $J = 7.0$, 6.5 ; δ_C 36.6); H-4, in turn, is correlated with Me-14 at

0.98 (d, $J = 7.0, 18.9$), which indicates that Me-14 is linked at position 4. The proton at position 3 coupled with the methylene group at 5.18 (dd, $J = 13.0, 4.0; 59.3$) indicates the attachment of a $-\text{CH}_2-$ group at position 3, which is further substantiated by long-range coupling with H-3 in HMBC spectrum. The assignments of the above protons were also confirmed by long-range coupling in the HMBC spectrum, wherein H-1 is correlated with C-10 (127.2), C-9 (144.3), C-5 (131.4) and C-3 (68.3); H-2 with C-1, C-3, C-4 and C-10; H-3 with C-2, C-1, C-5 and Me-14; H-4 with C-3, Me-14, C-5, C-10 and C-2; and Me-14 with C-4, C-5 and C-3, which are in accordance with the proposed structure of ring A. The OMe-15 at $\delta_{\text{H}} 3.79$ ($\delta_{\text{C}} 61.9$) shows long-range correlation with C-9 and C-8 (144.2) and hence it can be placed at position 9 of ring B. The hydroxyl proton at $\delta_{\text{H}} 8.07$ displays long-range coupling with C-12 (150.1) and C-8 (144.2) and hence it can be placed at position 12 of the ring C. The other hydroxyl proton at 1.59 exhibits correlations with C-6 (147.3) and C-7 (127.4), allowing it to be placed at position 6. The ^1H NMR spectrum has a three-proton singlet at $\delta_{\text{H}} 2.54$ ($\delta_{\text{C}} 31.9$) due to the methyl group at position 16 that displays strong long-range correlation with the carbonyl group at (204.7) assignable at position 13, C-11 (126.6) and a weak correlation with C-7, indicating an acetyl group attached at position 11. In addition, C-12 (150.1) and C-8 (144.2) appear downfield, which indicates that the oxygen atom is linked with C-12 and C-8 of the furan ring C. The methylene group at position 17 displays long-range correlation with a ketonic group (167.0), assignable at position-1', which is also coupled with Me-5' at $\delta_{\text{H}} 1.79$ (t, $J = 1.5$ Hz; $\delta_{\text{C}} 20.6$), and Me-4' at 1.91 (ddd, $J = 1.5, 1.5, 6.0$ Hz; 139.5). The small J values of 1.5 Hz indicate the *trans*-(or *Z*)-configuration of methyl groups [10]. Moreover, the proton at 6.03 (dd, $J = 1.5, 1.5, 6.0; 15.9$ Hz), attributable to position 3' shows long-range correlations with Me-5', Me-4' and C-2' (127.6), whereas Me-5' correlates with C-2', C-3' and CO-1'; and Me-4' with C-2', C-2' and CO-1', indicating that a methyl (2*Z*)-2-methylbut-2-enoate or methyl angelate is group attached at position 3 of ring A.

Thus, on the basis of above spectral analysis the structure of compound **3** is established as (6,12-dihydroxyl-9-methoxyl-4-methyl-11-acetyl-3,4-dihydronaphtho[2,3-*b*]furan-3-yl) methyl (2'*Z*)-2'-methylbut-2'-enoate, designated asirensane-c.

The other compounds were also characterized by spectral studies and compared with the reported values, whereupon compound **4** was identified as 9-methoxyl-4,11-dimethylnaphtho[2,3-*b*]furan, named as 14-nordehydrocalohastine, which has been reported previously from *Senecio linifolius* [6], whereas compound **5**, 4,11-dimethylnaphtho[2,3-*b*]furan-6,9-dione (designated as maturinone), has been isolated previously from *Cacalia decomposita* [7].

Anti-inflammatory Activity

The results are presented in Table II. The alcoholic extract decreased edema by 22% at a dose of 500 mg kg^{-1} after 3 h with respect to the control group treated only with carrageenan, while the standard drug phenylbutazone showed a 50% decrease at a dose of 100 mg kg^{-1} , indicating that the extract has moderate anti-inflammatory activity.

EXPERIMENTAL

General Experimental Procedures

Melting points were determined on a Metler 9100 Electro thermal apparatus by open capillary method and are uncorrected. IR spectra were recorded as KBr pellets on a UNICAM spectrophotometer, UV spectra on a Shimadzu UV-1601 UV-Vis Spectrophotometer, optical rotation on a Perkin-Elmer 241 MC Polarimeter, and mass spectra on a Finnegan MAT 300 mass spectrometer. The ^1H (500 MHz) and ^{13}C and DEPT 90 and 135

TABLE II Effect of alcoholic extract of aerial parts of *Senecio asirensis* on right rat paw swelling induced by carrageenan

Group (n = 6)	Dose (mg kg ⁻¹) orally	Carrageenan-induced edema					Mean increase in paw volume after 3 h	(% Inhibition)
		0 h	1 h	2 h	3 h			
Control (only carrageenan)	0.05 mL	0.93	1.53	1.64	1.82	1.82	0.88	-
Alcoholic extract	500 mg	0.93	1.50	1.58	1.62	1.62	0.69	22
Phenylbutazone (standard)	100 mg	0.94	1.20	1.32	1.39	1.39	0.44	50

NMR (125 MHz) and 2D NMR (COSY, TOCSY, HMBC, HMQC and NOESY) were recorded on a Bruker DRX 500 spectrometer in CDCl_3 using TMS as internal standard; chemical shifts δ are in ppm, and coupling constants (J) are in Hz. Elemental analysis was performed on a Perkin-Elmer CHNSO analyzer, model no. 2400. Column chromatography was performed using silica gel (0.04–0.063 mm, 230–400 mesh) as adsorbent. Centrifugal preparative TLC was carried out on a Chromatotron of Harrison Research Inc., USA, using a 4 mm rotor and silica gel PF-254 with $\text{CaSO}_4 \cdot 1/2 \text{H}_2\text{O}$ as an adsorbent. TLC was performed on silica gel 60 F-254 Merck plates and sprayed with vanillin– H_2SO_4 reagents for visualization of the spots.

Plant Material

The aerial parts of *Senecio asirensis* were collected on 21 April 2002 from wadi Al-Suda, Abha, Saudi Arabia and identified by a taxonomist of the center, where a voucher specimen No. 14488 has been deposited at the herbarium of College of Pharmacy, King Saud University for future reference.

Extraction and Isolation

The dried aerial parts (1.5 kg) were crushed to a coarse powder and extracted exhaustively with 95% alcohol in a percolator. The alcoholic extract was then concentrated and dried under reduced pressure to give a viscous mass (96.0 g) that was then fractionated into hexane-, chloroform- and methanol-soluble portions. The former two were mixed since they were almost similar on TLC (50.0 g); the solvent was evaporated, then the residue was dissolved in boiling methanol, kept overnight at room temperature, and filtered to remove fatty material (15.0 g). The filtrate was concentrated and chromatographed on a column of silica gel, successively eluted with hexane, ethyl acetate and methanol in increasing order of polarity. The fractions obtained from hexane–chloroform (9:1), (7:3), (3:7) and (1:9) were further purified separately by chromatotron using acetone–hexane as eluting solvent, which afforded asirensane-a (**1**) (80 mg), asirensane-b (**2**) (15 mg), asirensane-c (**3**) (22 mg), 14-nordehydrocalohastine (**4**) (150 mg), and maturinone (**5**) (150 mg).

Asirensane-a (**1**)

Asirensane-a (**1**) was obtained as light yellow needles (80 mg), eluted from the column with hexane–chloroform (1:2); mp 124–25°C, $[\alpha]_{\text{D}} -3.75$ (c 0.04, CHCl_3); R_{f} 0.44 (chloroform–hexane = 1:1); UV (CHCl_3) λ_{max} (nm) ($\log \epsilon$): 350 (3.11), 333.4 (3.13), 254.2 (1.29); IR (KBr) ν_{max} (cm^{-1}): 3450 (OH), 3115, 3074 (aromatic-CH), 2932 (Me), 2870 (CH_2), 1631, 1602, 1504 (C=C), 1461, 1410, 1370, 1343, 1247, 1220, 1166, 1033, 981, 811, 779, 685; HRMS: m/z (M^+ 256.1095) (calcd. for $\text{C}_{16}\text{H}_{16}\text{O}_3$ 256.1099). Elemental analysis: found (%): C 74.92, H 6.27, O 18.71; required for $\text{C}_{16}\text{H}_{16}\text{O}_3$: C 74.98, H 6.29, O 18.73.

Asirensane-b (**2**)

Asirensane-b (**2**) was obtained as light yellow needles (15 mg), eluted from column by chloroform–ethyl acetate (1:1); mp 162–63°C, $[\alpha]_{\text{D}} -26.67^\circ$ (c 0.03, CHCl_3); R_{f} 0.47 (water–methanol = 3:7, on RP C-18); UV (CHCl_3) λ_{max} (nm) ($\log \epsilon$): 313.6 (2.96), 276 (1.59), 240.8 (1.95); IR (KBr) ν_{max} (cm^{-1}): 3450 (OH), 3116, 3075 (aromatic-CH), 2930 (Me), 1745 (C=O), 1630, 1600, 1505 (C=C), 1460, 1410, 1370, 1344, 1245, 1222, 1160, 1030, 981, 840, 798, 745, 670; HRMS: m/z (M^+ 302.1152) (calcd. for $\text{C}_{17}\text{H}_{18}\text{O}_5$ 302.1154).

Elemental analysis: found (%) C 67.50, H 6.10, O 26.46; required for C₁₇H₁₈O₅: C 67.54, H 6.00, O 26.26.

Asirensane-c (3)

Asirensane-c (3) was obtained as a semi-solid (22 mg), eluted from the column with chloroform–ethyl acetate (1:2); [α]_D –77.5 (c 0.02, CHCl₃); R_f 0.48 (water–methanol = 3:7, on RP C-18); UV (CHCl₃) λ_{\max} (nm) (log ϵ): 310 (3.56), 282.2 (2.21), 244 (3.71); IR (KBr) ν_{\max} (cm⁻¹): 3445 (OH), 3115, 3075 (aromatic-CH), 2930 (Me), 1745, 1750 (C=O), 1632, 1605, 1505 (C=C), 1460, 1412, 1370, 1345, 1245, 1220, 1163, 1033, 980, 838, 798, 740, 683; HRMS: *m/z* (M⁺ 400.1520) (calcd. for C₂₂H₂₄O₇ 400.1522). Elemental analysis: found (%) C 65.91, H 6.02, O 27.95; required for C₂₂H₂₄O₇: C 65.99, H 6.04, O 27.97.

Experimental Animals

Wistar rats of either sex, weighing 200–250 g, were used to determine the anti-inflammatory activity. The animals were maintained at 23 ± 2°C with a 12 h light-dark cycle, fed a Purina rat chow diet supplied by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia, and had free access to food and water.

Determinations of Anti-inflammatory Activity

Six rats each were allotted to different treatment groups. Edema was induced in the rats by injecting carrageenan (0.05 ml, 1% w/v in normal saline) into the sub plantar tissue of the right hind paw using the method of Winter *et al.* [11]. Paw volume (ml) was measured with a plethysmometer (7140, Ugo Basile) before carrageenan injection and 0, 1, 2, and 3 h thereafter. The edema was reported as the difference between the initial and final volume. The anti-inflammatory effect was expressed as the percentage inhibition compared with vehicle-treated animals with respect to a reference group treated with phenylbutazone (100 mg kg⁻¹). The extract (500 mg kg⁻¹) with distilled water (0.1 mL per 100 g rat) was administered orally 1 h before injection of the phlogistic agent.

Acknowledgements

The authors are thankful to Mr Mukhair and Mr Ali Avvad for technical assistance.

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