Bull. Pharm. Sci., Assiut University
Vol. 7 part 2, pp. 389 - 497

PHYTOCHEMICAL STUDIES ON ASTER SQUAMATUS L.

PART II: Sesquiterpene Lactones, Triterpenes and Sterols present in the flowers.

S.A. Ross, H.M. Sayed and S.M. El-Sayyad
Department of Pharmacognosy, Faculty of Pharmacy, University Assiut, Assiut, Egypt.

ABSTRACT

Two sesquiterpene lactones santamarin and reynosin were isolated from the flowers of A.squamatus L. In addition α-and B-amyrin, ursolic acid, a mixture of stigmasterol, campesterol and B-sitosterol and an unidentified sesquiterpene lactone were isolated in pure form.

INTRODUCTION

Reviewing the current literature nothing could be traced dealing with the study of the constituents of A.squamatus L. In a previous publication[1], the flavonoid contents of the flowers of the title plant were recorded. We herein the isolation and identification of sesquiterpenes, triterpenes and sterols present in its flowers. This paper appears to be the first report of the isolation of the tricyclic sesquiterpene lactones santamarin and reyonosin from Aster species.

EXPERIMENTAL

M.P. are uncorr. IR-spectra were recorded in KBr and UV in EtOH. ^{1}H-NMR spectra at 100 or 60 MHz were recorded in $CDCl_3$ using TMS as internal standard and chemical shifts are given in ppm (δ). MS: direct inlet, 70 eV. Neutral alumina(Merck) was used for column chromatography. TLC and prep. TLC were done on silica gelG (Merck). Solvent systems used for TLC were: solvent 1: pet.ether ethyl acetate (9:1); solvent 2: pet.ether-ethyl acetate (85:15); Solvent 3: chloroform-methanol (59:5); solvent 4: chloroform; solvent 5: chloroform-acetone (9:1); solvent 6: pet.ether-chloroform-glacial acetic acid (15:25:0.5).

Plant Material:

The flowers of Aster squamatus L. were collected in December 1981 from plants growing wild on the River Nile banks in Assiut. The plant was identified and authenticated by Prof.Dr. N.El-Hadidi, Faculty of Science, Cairo University. The flowers were air-dried and powdered.

Extraction and Chromatographic Examination:

Two Kg. of the air-dried powdered flowers were extracted by percolation with EtOH 90% . The extract was concentrated and successively shaken with pet.ether and chloroform. Both the pet. ether and chloroform extracts were subjected to TLC examination.

Fractionation of petroleum-Ether Extract:

The pet.ether extract (25 g) was chromatographed over alumina column(1.5 kg. 120x10 cm) using solvent pet.ether, then pet.ether ethyl acetate mixtures in increasing polarities. Fractions(1 litre each) were collected and examined in TLC system 1. Five compounds were isolated and designated compounds 1-5. Their physical and chromatographic characters are given in the table.

Fractionation of Chloroform Extract:

The chloroform extract (18 g) was fractionatated over alumina column (600 g, 120x5 cm) using chloroform, chloroform methanol mixtures in increasing polarities. Fractions (400 ml) were collected and examined in TLC system 3. Two compounds were isolated and designated compounds 6 & 7 (the table).

Acetylation of Compounds 5,6 and 7:

20 mg sample of each compounds were separately mixed with 2 ml acetic anhydride and 0.5 ml pyridine and left overnight at room temperature then poured in acetone. The residue resulting after removing excess acetone and pyridine under vacuo was purified by TLC using system 5.

Compound 5 (santamarin):

White needles from $CHCl_3$ with m.p. 133-134°(lit.m.p. 134-135°) (2,3). UV λ_{max} 216 mu(log ε=3.833). IR ν_{max} cm^{-1} :3450 (sharp, OH), 2970 ($\geqslant$CH), 1770 (strong, α-methylene-γ-lactone). 1670 (C=O), 1440, 1345, 1270, 1135, 1080, 1045, 1010, 980,960, 860, 825. MS m/e (rel. int.): 248 $\{M\}^+$ (50), 233 $\{M-Me\}^+$ (5), 230 $\{M-H_2O\}^+$ (19), 220 $\{M-CO\}^+$ (8), 215 $\{230-Me\}^+$ (9), 152(77), 133 (32), 123 (31), 119 (40), 107{hydroxytropylium ion}(100),93 (50), 91 {tropylium ion} (60), 81 (64), 67 (34). ^{1}H-NMR at 100 MHz in $CDCl_3$: 0.87 (3H, S, C_{10}-Me), 1.82 (3H, brs, C_4-Me), 1.95-2.43 (6H,m, C_2-H, C_8-H and C_9-H), 3.62 (1H, dd, J=7 and 10 Hz. C_1-H) 3.93 (1H, t, J=10 Hz, C_6-H), 5.30 (1H, t, J=3Hz, C_3-H), 5.37 (1H, d, J=3Hz, C_{13}-Ha) and 6.05 (1H, d, J=3Hz, C_{13}-H_b). Santamarin acetate, m.p. 124-126° (lit. m.p. 125-128°) (4).

Compound 6 (reynosin):

White feathery needles from $CHCl_3$ with sharp m.p. 139-140° (lit. m.p. 143°) 95). UV λ_{max} 212 mu (log ε=4.061). IR ν_{max} Cm^{-1}:

3365(OH),3085,3010,2930,2870,2830,1760(strong,α-methylene-γ-lactone), 1665 (C=O), 1445, 1425, 1335, 1300, 1260, 1238, 1218, 1125, 1108, 1070, 1033, 995, 970, 945, 845, 812. MS m/e(rel. int.): 249 $\{M+1\}^+$ (12), 248$\{ M\}^+$ (61), 233 $\{M-Me\}^+$(6), 230 $\{M-H_2O\}^+$ (17), 220 $\{M-CO\}^+$(8), 215 $\{230-Me\}^+$ (10), 163 (34),152 L100), 151 (36), 133 (30), 123 (27), 119 (33), 109 (24), 107 (99.6), 105 (43), 95 (45), 93 (47), 19 (61), 81 (61) and 77 (45). ^{1}H-NMR at 60 MHz in $CDCl_3$: 0.93 (3H,s, C_{10}-Me), 1.90-2.55(8H,m, C_2-H, C_2-H, C_3-H, C_8-H and C_9-H), 3.58 (1H, dd, J=7 and 10 Hz, C_1-H),3.95 (1H, t, J=10 Hz, C_6-H), 4.84 (1H, brs, C_4=CH_2), 4.99 (1H, brs, C_4=CH_2), 5.42 (1H, d, J=3 Hz, C_{13}-Ha), and 6.08 (1H, d, J=3Hz, C_{13}-H_b). Reynosin acetate, m.p. 129-130°

Compound 7:

White amorphous powder from $CHCl_3$-CH_3OH mixture with m.p. 100-103°. UV λ_{max} 210 mu (rel. int. 83%). IR ν_{max} cm^{-1}: 3320 (broad, OH), 3080, 2935, 2900, 1765 (γ-lactone), 1605 (C=C), 1440, 1360, 1350, 1310, 1275, 1175, 1070, 1050, 1020, 1010, 855, 795. Acetate derivative , colourless gum.

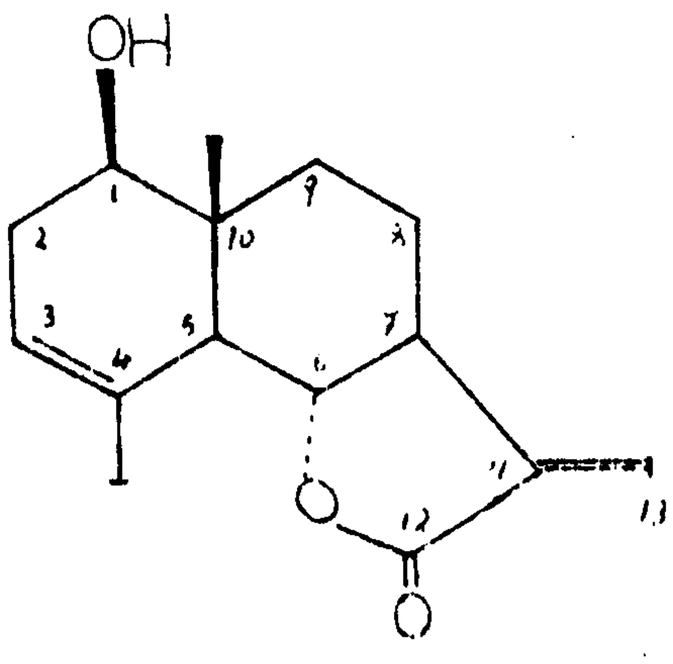
RESULTS AND DISCUSSION

Two kg. of the air-dried powdered flowers of A. squamatous L. were extracted with ethanol on cold and the concentrated extract was fractionated with petroleum-ether and chloroform. Both the petroleum-ether and chloroform extracts were separately fractionated over alumina columns to give seven pure compounds designated compounds 1-7

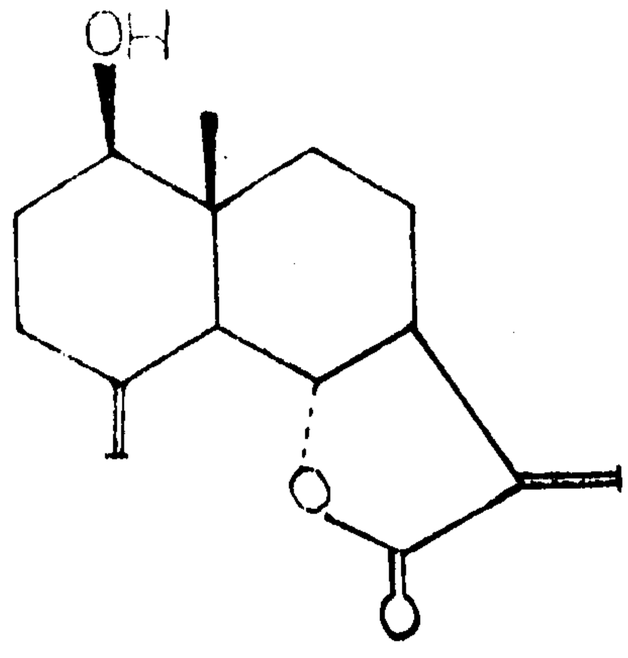
On the basis of co-chromatography, mixed m.p., physical properties, chemical tests and preparation of acetate derivatives, the first four compounds were proved to be B-amyrin (1),α-mayrin (2), B -sitosterol (3) and ursolic acid (4). TLC of the acetate

derivative of compound (3) on argentized silica gel G wedge shaped plates using system 6 proved to be a mixture compound of spots with R_f 0.23, 0.31 and 0.35 coinciding respectively with campesterol, stigmasterol and B-sitosterol acetates. Therefore, compound (3) is a mixture of campesterol, stigmasterol and B-sitosterol.

The physico-chemical properties and spectroscopic data(UV, IR, ^{1}H-NMR and Mass) of compounds 5 and 6 compare favourably with those published for tricyclic sesquiterpene lactones santamarin (2,3,4,6)and reynosin (2,5). Referring to the literature, it appeared that two isolated sesquiterpene lactones have cytotoxic activity in the Eagles carcinoma of the nasopharynx cell culture system (5) and isolated for the first time from plants of the genus Aster.



Santamarin



Reynosin

Compound 7 was isolated in small amount (36 mg) and has m.p. 100-103^{o}and maximum absorption in UV at 210 mu. The IR-spectrum showed a broad absorption band at 3320 cm^{-1} characteristic for

hydroxyl groups and a strong band at 1765 cm^{-1} corroborated the presences of a γ-lactone moiety. The acetate derivative is colourless gum. Insufficient material prevented a more detailed investigation. Work is in progress to isolate it again.

ACKNOWLEDGEMENTS

The authors express their gratitude for Dr. K.J.Harkiss (School of Pharmacy, Bradford University, UK) and Dr.A.M. Abdel-Aleem (college of Pharmacy , Florida University, U.S.A.) for spectroscopic assistance.

The physical chromatographic characters of the isolated compounds

Compounds	*$^{h}R_f$ in Systems*				*Colour with H_2SO_4*	*M.P. ^{o}C*	*Acetates m.p. ^{o}C*	*Amounts isolated in mg*	*Identification*
	1	*2*	*3*	*4*					
1	71	90			Reddish-brown	198-199	201-203	85	B-amyrin
2	33	60			Reddish-brown	184-186	225-227	282	∝-amyrin
3	18	48			Violet	135-138	125-127	168	mixture of stigmasterol, campasterol and B-sitosterol
4	10	30			Blue	277-279	-	20	Ursolic acid
5	5	12			pinkish-violet	133-134	124-126	135	santamarin
6			63	15	Violet	139-140	129-130	243	reynosin
7			28	8	Green	100-103	colourless gum	36	Unknown sesquiterpene

: System 1: pet.ether-ethyl acetate (9:1); System 2: pet.ether-ethyl acetate (85: 15);
System 3: Chloroform-methanol (95:5);
System 4: Chloroform.

REFERENCES

1) S.A. Ross H.M. Sayed and S.M. El-Sayyad; Egypt. J.Pharm. Sci., under publication.

2) H. Yoshioka, W. Renold, N.H. Fisher, A. Higo and T.J.Mabry; Phytochemistry, 9, 823 (1970).

3) H. Yoshioka, T.J. Mabry and B.N. Timmermann; Sesuiterpene Lactones; Tokoyo, University Press (1973).

4) I. Ognyanov and M. Todorova; Planta medica; Vol. 48 pp. 181-183 (1983).

5) M. Ogura, G.A. Cordell and N.R. Farnsworth; Phytochemistry; Vol. 17, pp. 957-961 (1978).

6) A. Romo de Vivar and H. Jimenez; Tetrahedron; 21, p. 1741 (1965).

الدراسه الكيمائيه لنبات الأستراسكوا ماتس ل ٠
الجزء الثانى ـ اللاكتونات السسكوترينيه والتربينات الثلاثيه والأستيرولات الموجوده فى الأزهار

سمير أنيس روس ـ هناء محمد سيد ـ ساميه محمد الصياد
قسم العقاقير ـ كلية الصيدله ـ جامعة أسيوط

تمكن الباحثون من فصل مادتين من لاكتونات السسكويتربين وهما السنتامارين والرينوزين ، أولا من الخلاصه البترواثيريه والثانى من خلاصة الكلوروفورم بعد تجزئتهما على أعمده من أكسيد الألومنيوم ٠

وقد تم التعرف على هاتين المادتين بالدراسات الفيزيائيه والكيمائيه والطيفيه لهما ومقارنة النتائج بتلك المنشوره من قبل ٠

وبالأضافه الى ذلك فصلت مواد أخرى معروفه وشائعة وهى كالآتى : ـ الفاوبيتا اميرين ، مزيج من الأسترولات غير المشبعه عبارة عن ستجما ستيرول، كامبا سترول وبيتا سيتو ستيرول وكذلك حمض الأورسوليك من خلاصة الأثير البترولى ومادة غير معروفه لها خواص السسكويتربين من خلاصة الكلوروفورم وقد تم التعرف على المواد السابقه عن طريق الدراسات المقارنه لمواد أصليه ٠

received in 5/3/1984 & accepted in 3/5/1984