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PHYTOCHEMICAL STUDIES ON ASTER SQUAMATUS L.
PART III: CONSTITUENTS OF THE LEAVES.

H.M. Sayed and S.A. Ross

Pharmacognosy Department, Faculty of Pharmacy,

Assiut University, Assiut, Egypt

ABSTRACT

From the air- dried powdered leaves of Aster squamatus L., we isolated and identified quercetin-3-methyl ether, quercetin-4'-methyl-ether, Kaempferol, quercetin, quercetin-3-O- diarabinoside, quercetin-3-methyl ether-4'-O- dirhamnoside, a saturated hydrocarbon, α-and β-amyrin, mixture of stigmasterol, campesterol, and β-sitosterol, sitosterol-3-O-xyloside, in addition to a triterpene alcohol and a triterpene acetate.

INTRODUCTION

Previously, these authors described the isolation and identification of squamatin, ternatin, rhamnetin, kaempferol, baicalein, luteolin-7-methyl ether and quercetin from the flowers of Aster squamatus L. (Asteraceae)[1]. Besides, two sesquiterpene lactones: santamarin and reynosin; as well as α-and β-amyrin,

ursolic acid, mixture of stigmasterol, campesterol and β-sitosterol were also isolated from the flowers of the same plant[2].

The present contribution reports on the isolation and identification of the leaves constituents of the title plant.

EXPERIMENTAL

General Experimental Procedures:

Melting points were uncorrected. All UV-spectra were in MeOH and IR spectra were in KBr discs. ^{1}H-NMR spectra were in $CDCl_3$ or DMSO-d_6 at 200MHz and chemical shifts are given in δ values. Mass spectral measurements were at 70 eV. Column chromatography was on silica gel, Merck or neutral aluminium oxide, Prolabo. Silica gel G 254, (Merck) and cellulose powder (Merck) were used for TLC. Whatmann paper 3MM was used for preparative PC. Acetylation was done by acetic anhydride/ pyridine method[3].

Plant Material:

The leaves of Aster squamatus L. Were collected in December 1981 from plants growing wild on River Nile banks in Assiut. The plant was identified and authenticated by Prof. Dr. N. El-Hadidi, Faculty of Science, Cairo University, Cairo. The leaves were air-dried, reduced to No 40 powder and kept in well-closed dark containers.

Solvent Systems:

Solvent I	: Petroleum ether-ethyl acetate	(9:1)
Solvent II	: Chloroform-methanol	(95:5)
Solvent III	: Chloroform-pet. ether	(1:1)
Solvent IV	: 40 % glacial acetic acid	

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Solvent V	**: Chloroform-glacial acetic acid-water**	**(50:45:5)**
Solvent VI	**: Chloroform-methanol**	**(9:1)**
Solvent VII	: Chloroform-methanol	(3:2)
Solvent VIII	: 15 % glacial acetic acid	
Solvent IX	: Chloroform-methanol-water	(50:13:1)
Solvent X	: n-butanol-pyridine-water	(6:4:3)
Solvent XI	: petroleum ether-chloroform-glacial acetic acid	(15:25:0.5)

Extraction:

One Kg. of the air-dried powdered leaves was successively extracted by percolation with pet. ether, chloroform and ethyl alcohol 70 %. The concentrated extracts were subjected to the following:

A- Petroleum-ether Extract:

The pet. ether extract (20 g.) was chromatographed over alumina column (600 g., 150 x 5 cm) using solvents pet. ether, pet. ether-ethyl acetate mixtures in increasing polarities. Fractions (500 ml each) were collected and subjected for TLC study using systems I and II.

Four compounds were isolated and designated compounds 1-4. Their physical and chromatographic characters are given in Table 1.

Compound 1 :

^{1}H-NMR in $CDCl_3$ showed : δ 0.88 (3H, t, J=6Hz), 1.26(S), 1.57(S). MS showed: M^+ at m/z 436(0.25), 422(0.07) (M^+-14), 408(0.4), 394(0.1), 380(0.22) 366 (0.08) , 352(0.11), 337(0.31), 323(0.36), 309(0.41), 295(0.50), 281(0.61), 267(0.73), 253(0.86), 239(1.06), 225(1.30), 211(1.62), 197 (2.02), 183(2.62), 169 (3.42), 155(4.49), 141(6.07) , 127(8.42), 113 (12.22), 99(18.65), 85(56.98), 71(79.10), 57(100), 43(37.67).

B- Chloroform Extract:

The chloroform extract (14 g.) was fractionated over silica column (500 g., 100 x 5 cm) using solvents pet. ether-chloroform (1:1), chloroform and chloroform-methanol in increasing polarities. Fractions (200 ml each) were collected and examined by TLC in systems II and III. From the first fractions we could isolate 2 compounds designated 5 and 6 (Table 2). The polar fractions showed the presence of flavonoidal components, which were separated and purified over preparative TLC and PC using solvent systems II and IV. We succeeded in the isolation of 4 flavonoidal compounds 7-10 (Table 3).

Compound 5:

It gave positive Liebermann- Burchard's[4] test. IRγ: 2950, 1725 (for ester linkage), 1645, 1460, 1370, 1250, 1130, 1075, 1020, 890, 980 cm^{-1}. ^{1}H-NMR in $CDCl_3$ showed δ: 0.85 (12H, S), 0.88 (3H-S), 0.93 (3H, S), 1.002 (3H, S), 1.02 (3H, S), 2.05 (3H, S, acetate protons), 4.61 (1H, d, J=1.7Hz). MS showed M^+ at m/z : 468 (10.8), 408 (4.1) $(M-CH_3COOH)^+$, 393 (4.1) $(M-CH_3COOH,-CH_3)^+$, 249 (13.26), 248 (0.76), 218 (10.17), 207 (1.65), 203 (18.9), 190 (34), 189 (100), 175 (20), 161 (18.98), 147 (23.65), 135 (50), 121(56.61), 109 (58.94), 107 (45.66), 95 (61.58), 81 (50.07), 69 (46.58), 67 (23.57), 55 (35.25), 43 (88.04).

Compound 6:

It gave positive Liebermann-Burchard's test[4]. IRγ: broad peak at 3280 (OH), 2880, 1640, 1470, 1450, 1390, 1300, 1120, 880 cm^{-1}. ^{1}H-NMR in $CDCl_3$ showed: δ 0.77(3H.S), 0.85(6H.S), 0.93(3H-S), 0.97(6H-S), 1.0(3H-S), 1.02 (3H-S), broad singlet at 4.61(1H). MS showed M^+ at m/z 426(28.1), 411(4.5) $(M-CH_3)^+$, 408(2.9)$(M-H_2O)^+$, 357(7.57), 315(6.36), 272(6.18), 229(5.87), 219 (11.28), 218(41.30), 207(69.1), 204(17.93), 203(27.04), 191(29.59), 190.5 (31.89), 189.5(79.34), 187.5(13.17), 175.5(24.24), 163(15.89), 161(24.45),

147(30.40), 137(18.44), 136(30.69), 135(73.62), 121(76.05), 109(85.19), 107(72.49), 95(100), 81(91.09), 69(84.32), 67(55.3), 55(90.72), 43(60.8).

Compound 7:

MS showed M^+ at M/z 316(98) and characteristic peaks at 301(8.1) $(M-CH_3)^+$, 285(8.2)$(M-OCH_3)^+$, 164(6) and 153(30). ^{1}H-NMR in $CDCl_3$ showed δ: 4.04(3H,S) for OCH_3), 6.46(1H,d, J=1.6Hz, H-8), 6.34(1H,d, J=1.6Hz, H-6), 7.05(1H,d, J=8.5Hz, H-5'), 7.75(1H,d, J=8.5, H-6'), 7.79(1H,S, H-2').

C- Ethyl alcohol Extract:

The ethyl alcohol extract (29 g.) was chromatographed over silica gel column (800 g., 150 x 5 cm) using solvents chloroform, chloroform-methanol mixtures in a manner of increasing polarities. Fractions (300 ml each) were collected and examined in the systems VI and VII. Fractions containing flavonoids were subjected to more purification on TLC and PC using solvent systems VII and VIII respectively, to give two compounds, labelled 11-12 (Table 4). Also during process of isolation we could isolate one white crystalline compound no. 13.

Compound 13:

m.p. 280-82°C, TLC, silica gel G, hR_f 40 and 68 in systems VI and IX respectively. It showed violet colour with H_2SO_4 spray reagent and gave positive Liebermann-Burchard's test[4]. IRγ: broad band at 3350-3480 (OH), 2970 (-CH), 1645(C=C), 1460, 1365, 1255, 1165, broad band at 1025-1080 cm^{-1}. Acid hydrolysis yielded aglycone (compound 14) and sugar xylose.

Compound 14 (sitosterol):

TLC, silica gel G, hR_f 61 system (VI) coinsiding with authentic sitosterol. It showed violet colour with H_2SO_4 spray reagent and gave positive Liebermann-Burchard's test[4]. ^{1}H-NMR in $CDCl_3$ and CD_3OD showed: signals

for 6 methyl groups in the region between 0.6-1.169 ppm, and a signal at 5.28 ppm (1H, distorted triplet, olefinic proton). MS showed M^+ at m/z 414 (0.89), 396 (2.10) $(M-H_2O)^+$, 381(0.87) $(M-33)^+$, 275(0.44), 273(0.98 (M-S.C.), 213(2.95) $(M-60-S.C.)^+$.

Acid Hydrolysis:

Each isolated glycoside was separately dissolved in N/2 H_2SO_4, mixed with an equal volume of ethanol and refluxed for 2 hours. The aglycones were extracted with ether, purified and subjected for TLC and spectral studies. The sugar moieties in the hydrolysates were examined on PC alongside with authentic samples using system X.

Mild Acid Hydrolysis:

Ten mg. of each glycoside were separately dissolved in N/10 H_2SO_4 (10 ml), mixed with an equal volume of ethanol and refluxed for 2 hours. A sample of the hydrolysate was withdrawn every 5 minutes during the first 20 minutes, then every 10 minutes during the remaining period and spotted on PC (3MM) using system VIII。

RESULTS AND DISCUSSION

The air-dried powdered leaves of Aster squamatus L. were successively extracted with petroleum ether, chloroform and ethanol 70%. Each extract was subjected to chromatographic studies.

By chromatographing the petroleum ehter extract over alumina column, four compounds were isolated (Table 1). Compound 1 has m.p. 58-60°C and its IR spectrum exhibited no special functional groups. The mass spectrum showed successive fragmentation of

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CH_2[5], indicating its paraffinic nature. The ^{1}H-NMR spectrum showed a sharp singlet at δ 1.26, δ 1.57 and triblet at δ 0.88 (J=6Hz) which confirmsits paraffinic nature. Therefore compound 1 is composed of saturated long chain hydrocarbon (s). Further gas chromatographic analysis is required to determineits exact composition.

On the basis of co-chromatography, mixed m.p., physical properties, chemical tests, acetate formation and comparison of IR spectra, compounds 2,3 and 4 were found to be β-amyrin, α-amyrin and β-sitosterol respectively. TLC examination of the acetate derivative of compound 4 on wedge shaped plates of argentized silica gel G using system XI revealed a mixture of campesterol, stigmasterol and β-sitosterol respectively.

Fractionation of the chloroformic extract over silica gel column followed by preparative TLC or PC, afforded six compounds (5-10). Four of them (7-10) were proven to be flavonoidal aglycones. Their chromatographic characters and spectral data, compare favourably with those published for quercetin-3-methyl ether[6,7], quercetin-4'-methyl ether[7], keampferol[6] and quercetion[6] respectively.

Compound 5 has m.p. 118-20°C and gave positive test for triterpenes[4]. The obtained purple colour with Liebermann's-Burchard test confirms that it is a triterpene. The MS showed M^+ at m/z 468 and peaks at m/z 248, 207 and 189 which are characteristic for triterpenes[9]. The ^{1}H-NMR spectrum showed sharp singlets in the region beteen 0.85-1.02 ppm which are integrated for 8 methyl groups, a sharp singlet at δ 2.05 (3H) indicative for acetate group and a signal at δ 4.61 which is attributed to CH-OR proton of the acctate function. The presence of the latter was also confirmed by the appearance of a peak in

MS at m/z 408 $(M-CH_3COOH)^+$ as well as a significant peak at 1725 cm^{-1} in the IR. Therefore, compound 5 is a triterpene acetate. Further spectral analysis is required to uncover its structure.

Compound 6 was isolated in the form of white needles with m.p. 156-58°C and showed positive test for triterpenes[4], the resulting purple colour indicates that it is a triterpene[8]. Its MS showed M^+ at m/z 426 and characteristic peaks for triterpenes[9] at m/z 248, 207 and 189. The appearance of a peak at m/z 408$(M-H_2O)^+$ indicates the presence of a hydroxyl group which is proven by broad band at 3280 cm^{-1} in the IR-spectrum. ^{1}H-NMR spectrum showed signal at δ 4.61 assigned for C<u>H</u>-OH proton. Signals corresponding for 8 methyl groups were also revealed at δ 0.77(3H, S), 0.85(6H, S), 0.93(3H, S), 0.97(6H, S), 1.0(3H, S) and 1.02(3H, S). Thus compound 6 is a triterpene alcohol. Further studies on both compounds 5 and 6 are in progress.

From the alcoholic extract after chromatography over silica gel column, three compounds were isolated and designated (11-13).

Compounds 11 and 12 gave positive tests of flavonoids[10]. Both, by mild acid hydrolysis, hydrolyze on two steps, indicating their biside nature. Acid hydrolysis gave aglycones which were proven by m.p., co-chromatography and UV-data to be quercetin and quercetin-3-methyl ether respectively. The sugar moieties were identified by PC to be arabinose for compound 11 and rhamnose for compound 12. Comparing the UV-spectral data (Table 4) with different complexing and ionizing agents for both the intact glycosides and their corresponding aglycones, it was proven that the sugars are attached to C-3 and C-4' respectively. Therefore, the structure for compounds 11 and 12 were suggested to be guercetin-3-O-diarabinoside and

quercetin-3-methyl ether-4'-O-dirhamnoside respectively. The obtained results for compound 11 compare favourably with that published for quercetin-3-diarabinoside which was isolated from Kalanchose pinnate (Fam. Grassulaceae)[11].

Compound 13 has m.p. 280-82°C and showed positive tests for steroids[4] and glycosides[12]. On acid hydrolysis, it yielded an aglycone which was proven by ^{1}H-NMR and MS to be sitosterol[13-15]. and a sugar moiety that was identified by PC to be xylose. Therefore, compound 13 can be considered as sitosterol-3-O-xyloside.

As far as we know, this is the first report of the isolation and identification of sitosterol-3-O-xyloside from genus Aster. Interestingly, there have been two reports of the isolation of sitosterol-3-O-xyloside from Bauhinia candicans[15] and Maytenus senegalensis[16].

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Table 1: Characters of the Compounds Isolated from the Pet.ether Extract

Compound No	Hr_f in Syst.* I	Hr_f in Syst.* II	Colour with H_2SO_4	M.P,C.°	Acetates M.P,C.°	Amounts isolated in mg.	Identification
1	80	93	Y.B.	58-60	---	20	Saturated Hydrocarbon(s)
2	70	88	R.B.	198-199	201-203	95	β-amyrin
3	32	61	R.B.	184-186	225-227	175	α-amyrin
4	17	49	V	135-138	125-127	190	Stigmasterol, campesterol and β-sitosterol.

*hR_f on silica gel G plates; R.B.: reddish brown, V.: violet, Y.B.: Yellewish brown

Table 2: Characters of Compounds 5 and 6 Isolated from the Chloroform Extract.

Compound No.	hR_f in Syst.* II	hR_f in Syst.* III	Colour with H_2SO_4	Amounts isolated in mg.	M.P,C.°	Identification
5	68	63	R.B.	32	118-120	Triterpene acetate
6	48	32	R.B.	15	156-158	Triterpene alcohol

*hR_f on silica gel G plates. R.B.: reddish-brown.

Table 3: Chromatorgraphic Properties and UV data for the Flavonoids 7-10 Isolated from the Chloroform Extract.

Compound	Amounts isolated in mg.	hR_f in systems V[x]	VI[xx]	Colours UV	Colours $AlCl_3$	m.p.°	UV λ_{max} in MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc	+NaOAc/H_3SO_3
7	15	90	70	P.	Y.	273-75	258,270* 356	258,270* 366(decomp)	278,342* 434	270,282*, 373	258,263* 366	256,376
8	22	56	65	D.Y.	Y.G.	259-60	254,270* 370	274,310* 384	270,300* 360,430	270,300* 360,430	268,276 322,400	270,310 372
9	150	50	41	Y.G.	Y.G.	278-81	252,266 320*,367	278,316 416(decomp)	260,268 350,420	258*,270 348,425	274,300 387	267,300* 320*,370
10	100	38	26	B.Y.	Y.G.	316-18	255,268*, 370	247*,321 (decomp)	270,304 330,459	265,300* 360,428	258*,274 330,390 (decomp)	260,303* 388

[x] hR_f on cellulose plates [xx] hR_f on silica gel G plates. * Shoulders P.: pink, Y.: Yellow, D.Y. dark yellow, Y.C.: Yellowish-green, B.Y.: Bright yellow.

Table 4: Physico-Chemical Characters as well as Spectral Data of Compounds 11-12.

Characters	Compound 11	Compound 12
m.p.	180°C Charring	200°C Charring
hR_f*	35	21
Amount isolated	150 mg.	100 mg.
Mild acid hydr.	two steps	two steps
Acid hydrolysis	quercetin + arabinose	quercetin-3-methyl-ether + rhamnose
UV-data,nm.		
MeOH	246, 300 sh, 340	256, 272, 338
+ NaOMe	274, 340,380	278, 388 (↓ in intensity)
+ $AlCl_3$	264, 306,358	276, 300 sh, 355, 402
+ $AlCl_3$/HCl	248 sh, 300, 340	278, 305 sh, 350, 398
+ NaOAC	256, 304, 354	276, 308 sh, 370
+ NaOAC/H_3BO_3	260, 304, 354	262, 362
IR (cm^{-1})	3240, 3500, 1700 1610, 1530, 1280	3240-3500, 1600, 1280
Identification	quercetin-3-O-diarabinoside	quercetin-3-methyl ether-4'-O-dirhamnoside.

*hR_f, TLC, silica gel G plates, system: VII.

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الدراسة الكيميائية لنبات الاسترسكوا ماتس ل ٠
الجزء الثالث : مكونات الاوراق

هناء محمد سيد ـ سمير انيس روس

قسم العقاقير ـ كلية الصيدلة ـ جامعة اسيوط

قام الباحثان باستخلاص اوراق نبات الاسترسكوا ماتس ل بالبترول الاثيرى والكلوروفورم والكحول بالتتابع ثم فصل ودراسة مكونات كل خلاصة على حدة والتعرف عليها بالطرق الفيزيائية والكيميائية ودراسة الرنين النووى المغناطيسى ومطياف الكتلة لها ٠

فمن خلاصة البترول الاثيرى تم التعرف على هيدروكاربون مشبع ، بيتا اميرين، الفا اميرين وخليط من ستجما ستيرول وكامبيستيرول وبيتا سيتوستيرول٠

ومن خلاصة الكلوروفورم تم فصل اربعة مركبات فلافونويدية حرة وهى : كورستين ـ ٣ ـ ميثيل ايثر ، كورستين ـ ٤ ـ ميثيل ايثر ،كامبيفيرول وكورستين بالاضافة الى تربين ثلاثى فى صورة خلات وآخر تربين ثلاثى كحولى ٠

اما الخلاصة الكحولية فقد فصل منها جلوكوزيدات فلافونويدية مثل كورستين ـ ٣ ـ أ ـ ثنائى الارابينوزوكورستين ـ ٣ ـ ميثيل اثير ـ ٤′ـ أ ـ ثنائى الرامنوز وستجما ستيرول ـ ٣ ـ أ ـ زيلوز ٠

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