POLYMETHOXYLATED FLAVONES FROM SOLANUM ABUTILOIDES, GROWN IN EGYPT (SOLANACEAE)

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ABSTRACT:

Two polymethoxyflavones,5-O-demethylnobiletin and 7,4'-dihydroxy-5,6,8,3'-tetramethoxyflavones were isolated from the chloroform extracts of fresh yellow-orange berries of Solanum abutiloides. The two compounds are reported for the first time in the genus Solanum. The light petroleum fraction, gave branched aliphatic alcohol, α-amyrin, lupeol, phytosterol and stigmasterol glucoside. Identification of these compounds was established on the basis of H-NMR, MS, UV, IR and comparison with authentic samples. GLC analysis of fatty acid methyl esters revealed the presence of 15 fatty acids, where linoleic, palmitic and oleic acids represented 37.54, 32.61 and 17.52%, respectively.

INTRODUCTION

Solanum is one of the largest genera in the plant kingdom. It includes about 1400 species widely distributed throughout the world(1, 2)

In recent years, the genus has been extensively screened for steroidal alkaloids and similar nonnitrogen-containing isoprenoides which have a potential value to steroid industry. Beside alkaloids, flavonoids form the other major group of compounds reported from Solanum genus. Thirty-eight different flavonol glycosides and one flavone glycosides were identified from the leaves of 107 Solanum species(3). Some Solanum species have been reported to contain methylated flavonoids (4-7). Methylated flavonoids, free or as glycosides were found to have cytotoxic properties (8-10). Thus, the objective of this research was the isolation and identification of flavonoids and other constituents which might have potential agrochemical activity in the fresh berries of Solanum abutiloides. To the best of knowledge, nothing has been reported on flavonoidal content of berries of this species.

Solanum abutiloides is a herbaceous bush native to Argentina but easily cultivated elsewhere. It produces clusters of fruits which on ripening change from dark green to yellow-orange. We cultivated this plant in the Experimental Station of Faculty of Pharmacy, Zagazig University in order to survey its constituents.

In former investigation, steroidal alkaloids, and spirostane isonuatigenin have been isolated from the fruit and root extracts of Solanum abutiloides (7,11-17) as well as flavonol and flavonol glycosides were isolated from ethyl acetate extract of the aerial parts(7). The aqueous extracts of Solanum abutiloides grown in Egypt, is effective in intestinal and uterine spasms and in treating bronchial asthma, it has hepatoprotective effect, but caution should be paid to its side effects on male fertility(18) Solanum abutiloides has potent antimicrobial activity for most Gram +ve organisms and fungi and inactive against Gram -ve bacteria (7) and it's root exudates showed anti-fungal activity(19).

This paper represents the first report for polymethoxy flavone from the genus Solanum.

EXPERIMENTAL

1- Plant material:

Provided seeds and the cultivated plant were identified as Solanum abutiloides Bitt et Lillo. Solanaceae by Prof. Dr. Darwish Ibrahim, Professor in Department of Horticulture of Efficiency Productive Institute, Zagazig University. Plant was grown in the Medicinal Plant Station of Faculty of Pharmacy, Zagazig University, Egypt. Fresh yellow-orange berries (1.5 kg) were collected in June 2003, Voucher specimens are retained in Medicinal Plant Station of Faculty of Pharmacy, Zagazig University, Egypt.

2- Apparatus:

Compact and Handheld Ultraviolet lamp, UVGL-55; Melting points were determined with a digital melting point apparatus, electrothermal, LTD, England and were uncorrected; UV spectra were recorded on a Cintra-5, UV-vis, spectrometer, Pty-LTd; IR were measured on a Perkin Elemer FT-IR1650 machine; Mass spectra were measured on a Shimadzu GC-MS, QP 5050A spectrometer, EI (70 ev); H-NMR spectra were recorded in CDCl3 on a Varian XL-200 spectrometer, at 200 MHz, chemical shifts are given in ppm with TMS as internal standard. Fatty acid methyl esters were analyzed on GC Pye Unicam gas chromatography under the following operating conditions: Detector: FID; Temperature of detector: 300°C; Temperature of injector: 270°C; Recorder: Dual channel recorder, Column temperature: 70°C-290°C (8°C/min);Column package for fatty acid methyl esters: Diatomite C (100-120 meshes);Liquid Adipate Glycol Polyethylene 10% phase: (PEGA);Column dimensions: 1.5 m x 4 mm; Carrier gas: Nitrogen 30 ml/min; Hydrogen flow rate: 33 ml/min; Air: 300 ml/min; chart speed; I cm/min.

Authentic fatty acid methyl esters were supplied by central research laboratory, Faculty of Agriculture, Cairo University.

3- Adsorbent:

- Silica gel 60 GF254 for TLC (Adwic Egypt).

- Precoated TLC sheets, silica gel 60 GF254 (Merck).
- Silica gel G (60-230 meshes, Merck) for column chromatography.

4-Solvent systems:

- System 1: Light pet.: EtOAc (30: 5).
- System 2: Light pet.: CHCl₃: MeOH (30: 15: 15).
- System 3: BuOH: HOAc: H₂O (4:1:5).
- System 4: Benzene:EtOAc (3:1).
- System 5: Light pet.: CHCl3: glacial HOAc (75;25;0.2).

Reference compounds: sterols, triterpenes, and from Department obtained sugars were of Pharmacy, Zagazig Pharmacognosy, Faculty University.

Extraction and isolation

Fresh yellow-orange berries (1.5 kg) were crushed in blender and exhaustively extracted with 95% ethanol. The solvent free semisolid extract (80 g) was suspended in 300 ml water and fractionated with light petroleum, chloroform and ethyl acetate to give 25 g, 5 g and 3.5 g of dried extracts respectively.

1- Column chromatography of the chloroform extract:

TLC examination of chloroform extract showed the presence of flavonoids, the spots were visualized by spraying with H2SO4 (50%) and heating at 110°C, as well as they gave yellow colour with NH4OH and AICI, (T.S).

The chloroform extracts (4 g) chromatographed on silica gel column (3 x 50 cm, 100 g) in benzene, elution was started with benzene and polarity was increased by ethyl acetate. Fractions eluted with 2% ethyl acetate in benzene afforded (35 mg) of flavonoid I, as yellow needles (from CHCl3-MeOH). Fractions eluted with 4% ethyl acetate in benzene afforded a mixture of flavonoid 2 and some minor compounds. PTLC of this mixture using solvent system 4 (double run), afforded 28 mg of compound 2 (Rf of 0.52) as pale yellow needle crystals (CHCl3-MeOH).

Compound 1: Yellow needles crystallized from (CHCl₃-MeOH) m.p of 140-142°C, UV (λ_{max} nm): (MeOH) 282, 340; (MeOH-NaOMe), 290 (sh), 316, 403 (sh), (MeOH-NaOAc), unchanged; (MeOH-NaOAc-H3BO3), unchanged; (MeOH-AlCl3), 285, 360, 410 (sh); (MeOH-AlCl₃-HCl), 286, 356, 410 (sh), EI-MS, m/z (rel. int. %) 388 [M⁺] (43), 373 (62), 211 (8), 183 (16), 165 (9), 163 (4), 162 (14), 69 (100); HNMR (200 MHz, CDCl₃) (Table 1).

Compound 2: Pale yellow needles, (CHCl3-MeOH); mp of 150-152°C,

UV (λ_{max}, nm): (MeOH) 280, 345; (MeOH-NaOMe) 268, 320 (sh), 408; (MeOH-NaOAc) 278, 346, 410 (sh); (MeOH-NaOAc-H₃BO₃), unchanged; (MeOH-AICl₃) 280, 362; (MeOH-AICl₃-HCl), 276, 361. EI-MS: m/z (rel. Int. %) 374 [M⁺] (72), 360 (20) 359 (100), 331 (3), 316 (3), 211 (18), 187 (4), 183 (28), 165 (9), 162 (1), 151 (14), 148 (26), 133 (51), 105 (42), 57 (50), 58 (4); HNMR (200 MHz, CDCl₃) (table 1).

Table (1): HNMR spectral data of flavones 1-2 (in CDCl₁, TMS as int. st, at 200 MHz)

CDCI3, TWIS as Int. st, at 200 1/11/2/					
H	1	2			
3	6.63 (s)	6.64 (s)			
2'	7.44 (d, 2.0 Hz)	7.43 (d, 2.0 Hz)			
6'	7.61 (dd, 8.6, 2 Hz)	7.60 (dd, 8.5, 2 Hz)			
5'	7.00 (d, 8.6 Hz)	7.05 (d, 8.5 Hz)			
3'					
OH-5	12.56 (s)	-			
OH-7					
OH-4'	-				
OMe	4.14 (s)	4.12 (s)			
	4.01 (s)	4.05 (s)			
	4.00 (s)	4.00 (s)			
	3.98 (s)	3.98 (s)			
	3.98 (s)				

2-Column chromatography of the light petroleum extract:

About 10 g of light petroleum soluble fraction was subjected to silica gel column chromatographic analysis (3 x 100 cm, 300 g).

Gradient elution technique was performed using light petroleum to which ethyl acetate was added in an increasing proportion. The effluent was collected in 200 ml fractions, and monitored by TLC (system 1). anisalaldehyde/sulphuric acid as a revealing agent and similar fractions were combined.

Compound 3:

Fractions eluted with 3% ethyl acetate in light petroleum, yield 35 mg of white granular powder (CHCl₃-MeOH), with R_f of 0.68 (system 1), mp of 78. 80°C and IRγ^{Kbr} cm⁻¹: 3318-3226, 2955, 2917, 2848, 1467, 1376, 1344, 1264, 1133, 1094 and 904; El-MS: M/Z (% relative abundance) 298 [(M⁺, 3) (calculated for Call Co. 139 for C₂₀H₄₂O)], 297(9), 185 (M⁺-C₈H₁₇, 16.12), 71 (2.46), 125 (6.62) (2.46), 125 (6.68), 111 (30), 97 (40), 83 (39.5), 71 (37.7), 57 (1000) (37.7), 57 (100%), 55 (63) and 53 (4)-

Compounds 4 and 5:

On TLC screening of fraction (25-34) eluted with EtOAc in light 5% EtOAc in light petroleum, they showed two major spots, R. value. spots, R_f values of 0.48 and 0.43 (system 1), was residue left and residue left after solvent distillation (600 mg), was rechromatograph rechromatographed on silica gel column (1 x 50, 30 f silica), 2% EtOAc in light petroleum as eluting system, 100 ml fractions were collected.

Fraction (7-22), showing single spot R_f of 0.48 (system I), 120 mg white needle shaped crystals (CHCl₃-MeOH), m.p of 186°C, gives positive results with Libermann's and Salkowski's tests (20,21) and $IR\gamma^{Kbr}$ cm⁻¹, 3395, 2946, 2870, 1611, 1459 and 1382, EI-MS: M/Z (rel. int %), a molecular ion peak (M⁺), m/z at 426 (2.8) (coinciding with molecular formula $C_{30}H_{50}O$), 411 (1.68), 393 (0.63), 257 (2.28), 218 (27.7), 207 (14), 205 (7.79), 204 (7.14), 203 (19.6), 189 (24.9), 149 (25), 107 (40) and 55 (100). This compound was designated as compound 4.

Fractions (30-39), showed single spot of Rf of 0.43 (system I), white needle shaped crystals of compound 5, mp. of 212-216°C, gives positive Libermann's and Salkowski's tests^(20,21). IR γ ^{Kbr}: 3356, 2937, 2869, 1708 and 1644, 1456, 1383, 1295, 1186, 1080, 1038 and 878; EI-MS M/Z (rel. int. %): 426 (M⁺ 7.3) (calculated for C₃₀H₅₀O), 411 (1.5), 408 (1%), 393 (1.1), 357 (2.8), 315 (2.8), 303 (0.31), 297 (2.34), 257 (3.2), 248 (0.47), 229 (4.33), 220 (2.9), 218 (10.27), 207 (37.6), 205 (8.69), 203 (11.97), 192 (2.95), 189 (54), 149 (18.3), 147 (23.7), 135 (51), 121 (69.4) and 95 (100%).

Compound 6:

Fractions eluted from 10% EtOAc in light petroleum, on evaporation and repeated crystallization (CHCl₃-MeOH), gave 50 mg, white shinny crystals, $R_{\rm f}$ of 0.33 (system I); $IR \gamma_{\rm max}^{KBr}$ cm⁻¹: 3865-3423, 2948, 2865, 1649, 1457, 1374 and 1055 cm⁻¹. EI-MS: M/Z (rel. int. %): 414 (M⁺, 34.8) ($C_{29}H_{50}O$), 412 (27.7) ($C_{29}H_{48}O$), 399 (12.7), 397 (7.4), 396 (12), 394 (3.7), 383 (2.3), 381 (10), 329 (14), 327 (1.5), 303 (12.5), 301 (5), 273 (15), 271 (20), 255 (36) and 213 (22.4).

Acetylation of compound 6(22,23):

Compound 6 (20 mg) was acetylated (22,23). TLC examination of acetyl derivative used silica gel G chromatophlats. Impregnated with 10% aqueous AgNO₃ (system 5) and antimony trichloride spray reagent (24), revealed two adjacent violet spots. The major corresponded to reference B-sitosterol acetate and the minor to reference stigmasterol acetate.

Compound 7:

Fractions eluted with 40% EtOAc in light petroleum, gave 100 mg of white scales pure compound (hot methanol), R_f values of 0.05 and 0.36 (systems 1 and 2) respectively, reacts positively with Libermann's and Salkowski's, Molish's tests and reducing Fehling's solution after acid hydrolysis.

IR $\gamma_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3731-3228, 2960, 2918, 2847, 1622, 1466, 1375, 1133 and 1059.

EI-MS: (M/z, rel. int.) 574 (0.99), 412 (1.19), 225 (1.19), 212 (1.19), 185 (4), 177 (2.3), 171 (3), 159 (0.99), 106 (3), 83 (14), 69 (44) and 55 (100%).

Acid hydrolysis of compound 7:

About 20 mg of compound 7 was dissolved in hot methanol and refluxed with 10 ml of 5% sulphuric acid for two hours in a boiling water bath. The mixture was cooled and extracted with chloroform (3 x 25 ml). The chloroformic extracts were washed with water, dried over anhydrous sodium sulphate and concentrated under vacuum. The residue was gave colourless needle shaped crystals of m.p of 138-140°C. Direct comparison with authentic sample of stigmasterol (m.p., m.m.p and Co. TLC) proved that the aglycone is stigmasterol.

The aqueous acidic solution remained was neutralized with barium carbonate and examined by paper chromatography against authentic sugars samples (system 3), visualized by aniline phthalate reagent yielded a single brown-spot with R_f value identical with that of glucose.

3- Saponification of the light petroleum extract and preparation of fatty acid methyl esters (22,25,26):

About 10 gm of the light petroleum extract was subjected to saponification gave (2.8 g) of the USM. The fatty acid methyl ester were prepared from the alkaline solution remained after extraction of USM and then subjected to GLC analysis. The identification of the investigated fatty acids was established by comparing the retention times with those of authentics. Quantitative estimation was carried out by peak area measurements followed by normalization.

Table (2): Results of GLC analysis of the fatty acid methyl esters

methyl esters						
Peak No	Carbon number and number of double bonds	Retention time	% of fatty acid	Components		
1	C8:0	3.27	0.065	Caprylic		
2	C9:0	4.5	0.017	Nonanoic		
3	C10:0	5.76	0.07	Capric		
4	C11:0	6.85	0.152	Hendecanoic		
5	C12:0	8.33	0.278	Lauric		
6	C13:0	9.54	0.103	Tridecanoic		
7	C14:0	10.77	2.478	Myristic		
8	C15:0	12.05	1.895	Pentadecanoic		
9	C16:0	13.25	32.61	Palmitic		
10	C17:0	14.11	0.86	Margaric		
-	C18:0	14.73	0.419	Stearic		
11	C18.1	15.44	17.523	Oleic		
12	C18.2	16.14	37.544	Linoleic		
13	C18.3	17.94	5,922	Linolenic		
14	C20.0	21.77	0.064	Arachidic		
15	C20.0	2.00				

RESULTS AND DISCUSSION

Repeated column chromatography of the chloroform extract followed by PTLC resulted in the isolation of the polymethoxyflavones (1-2). UV spectra which were carried out with diagnostic reagents using standard procedures (27,18), as well as the HNMR data revealed a great similarity between compounds (1-2). The UV spectra in methanol are typical for flavones or 3-substituted flavonols. The presence of the H-3 singlet at δ 6.63 in the HNMR excluded the presence of flavonols leaving flavones as the only possibility (28,29).

The MS of compound I exhibited a molecular ion indicating peak m/z 388 (43%)monohydroxypentamethoxyflavone (C20H20O8). The low UV shift (+16 nm) of band I upon addition of AICl₃ + HCl suggested a 5-hydroxyflavone which is oxygenated at C-6(19,30). The one hydroxyl was located on C-5 (chelated hydroxyl signal in the ¹HNMR at δ 12.5). The ¹HNMR showed a singlet proton at δ 6.63 assigned to H-3, five aromatic methoxyl groups at 8 4.14, 4.01, 4.00, 3.98 and 3.98 and aromatic protons showing a typical pattern for 3',4'-disubstitution at δ 7.6 (dd), 7.44 (d) and 7 (d) assigned to H-6', H-2' and H-5', respectively (28,30). All these facts together with the MS fragmentation (scheme 1), indicated that the five methoxyls are distributed over C-6, C-7, C-8, C-3' and C-4' of the ring system.

The previous data besides comparison with published data for similar polymethoxy flavones⁽²⁷⁻³⁵⁾ confirmed that compound I has to be 5-hydroxy-6,7,8,3',4'- pentamethoxyflavon (5-Odemethylnobiletin)^(32,34,35)

Compound 2 was isolated as pale yellow crystals. UV (280, 345 nm) absorptions are typical of flavones skeleton(28). The MS of 2 exhibited a molecular ion peak at 374 (71%) in accord with a dihydroxytetramethoxyflavone (C19H18O8). The base peak ion at m/z 359 is due to (M*-15) and the ion appeared at m/z 331 (M-43) are diagnostic fragmention pathway for 6and 8- methoxy flavones(28). Other MS fragments at 183 (scheme 1) indicated a m/z 211 and monohydroxy-trimethoxy substituted on ring A, while those at m/z 151 and 148 placed the second hydroxyl and the remaining methoxyl on ring B. The 'HNMR data (table 1) confirmed the presence of four methoxyl, an olefenic proton at δ 6.64 assigned to H-3 and three aromatic protons coupled with AMX system. At δ 7.43, 7.05 and 7.6 assignable to H-2', H-5' and H-6', respectively, confirming a 3',4'-dioxygenation in ring B. The UV spectrum of compound 2 on addition of NaOMe exhibited a shift (+63 nm) in band I, placing a hydroxyl group at C-4(28,31) and hence a methoxyl group must be located at C-3'. The absence of a free 5-OH is deduced: firstly from the absence of a chelated-OH signal in the 'HNMR, and secondly from the difference between the obtained data for compound 2 and those reported for 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone The UV spectrum (NaOMe) of 2 showed a new band at 320 nm (cf of MeOH) indicating a free 7-OH group From the previous data together with comparison with the enormous published data (9,34) for similar compounds, it was concluded that compound 2 is 7,4'-dihydroxy-5,6,8-3'-tetramethoxyflavone. These compounds (1-2) have not been reported in the genus Solanum before. However, 13CNMR assignment of these compounds will be addressed upon isolation of further quantity of them.

Column chromatography of the light petroleum extract gave an aliphatic alcohol compound 3, with molecular formula of C₂₀H₄₀O, low melting point, low polar character, IR, MS and reported data^{Cl4} confirm its structure.

Compound 4 was identified as a-amyrine, the identity was established by direct comparison (Co-TLC, m.p, IR and MS) with authentic samples as well as reported data⁽³⁷⁾.

Compound 5 showed atypical MS fragmentation pattern of lupeol (38,39), m.p of 212-216°C and COTLC with authentic lupeol confirm it is structure to be lupeol.

Compound 6 was identified as a mixture of β sitosterol and stigmasterol. However, the relative
intensity (%) of the indicated ions suggested the
presence of B-sitosterol as the major component. This
was also established by TLC examination of the acetyl
derivatives (this mixture is known as phytosterol).

Compound 7 was identified as stigmasterol glucoside through acid hydrolysis and direct comparison with authentic sample of sterol (CO-TLC, m.p, IR and MS), the sugar part was proved to be glucose (through TLC).

Lupeol, phytosterol and stigmasterol glucoside were previously isolated from Solanum species (40-43).

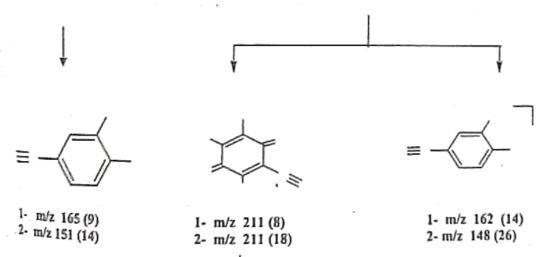
GLC analysis of fatty acid methyl esters (table 2), revealed that 15 fatty acids out of were identified, the total percentage of the identified fatty acid is 95%, the major one is linoleic acid (37.54%), then palmitic (32.61%) and oleic acid (17.52%) and the unsaturated fatty acid content (oleic, linoleic and linolenic) constitutes about 61% of the total fatty acids in Solanum abutiloides

The previous results indicate that Solanum abutiloides elaborates two of polymethoxyflavones. 5. O-demethylnobiletin I is currently being tested for its differentiation inducer activity for myeloid leukemic cells⁽⁹⁾. The presence of a free-OH group at C-7 in flavone 2 strongly suggests it as an effective polymethoxyflavone regarding inducing of leukemic cells (MI) to have phagocytic activity⁽⁹⁾.

- $R_1=H$
- $R_1=R_3=Me$ 2-
- $R_1 = R_3 = R_4 = Me$ $R_1 = R_4 = H$

- R₂ R₃ R_4
- H Me OMe Me: m/z 388 (43)
- Me H OMe H: m/z 374 (72)
- 1m/z 373 (62)
- 2m/z 359 (100)

2-331 (3)



- 1- m/z 183 (16)
- 2- m/z 183 (28)

Scheme (1): Selected mass spectral fragments of flavones 1-2(18)

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Zagazig J. Pharm. Sci., December 2006 Vol. 15, No. 2, pp. 53-59

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Received: Nov. 17, 2006 Accepted: Dec. 20, 2006

فلافونات علىداة الميثيل من الثمام الطازجة لنبات سولانمر ابيوتيلويداز المنزع في مص والنابع للعائلة الباذ فجانية

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تم فصل ٥-أ-س ميثيل النوبلتين و٧, ٤' ثنائى الهيدروكسى ٥, ٦, ٨, ٣' رباعى ميثيل الفلاقون لأول مرة من جنس السولانم من خلاصة الكلوروفورم للثمار الناضجة. ومن خلاصة الإيثير البترولى تم فصل كحـول اليفاتى وأربع مركبات استيرولية. وقد تم التعرف على التركيب الجزئى لجميع المركبات المفصولة بواسطة التحاليل الطيفية والطبيعة المختلفة. وكذلك تم التحليل الكيفى والكمى للأحماض الدهنية بواسطة كروماتوجرافيا الغاز.