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TRITERPENOIDS AND FLAVONOIDAL GLYCOSIDE FROM FRUITS OF *SORBUS AUCUPARIA* (DC) CULTIVATED IN EGYPT

S. A. Youssef

Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

يعتبر نبات سوربس اكيوباريا من النباتات التى تستعمل لعلاج كثير من الأمراض ونظرا لأهمية هذا النبات فى الطب الشعبى وعدم وجود دراسة كيميائية كافية عليه للتعرف على مكوناته الكيميائية فقد رؤى إجراء دراسة كيميائية لمكوناته المختلفة.

وقد أمكن فصل والتعرف على الفا اميرين ، بيتا سيتوستيرول ، حمض الأورسليك ، ميثيل ٢ ألفا هيدروكسى أورسلات ، ٢ ألفا هيدروكسى أورسلات من خلاصة الكلوروفورم وأمكن التعرف عليهم ، وتم فصل مادة الروتين من خلاصة خلات الإيثيل. وذلك بدراسة خواصها الطبيعية والكيميائية ومقابلتها لعينات أصلية وكذا الطرق الطيفية المختلفة مثل الأشعة فوق البنفسجية والأشعة دون الحمراء والرنين النووى المغناطيسى وكذا طيف الكتلة.

From the chloroform soluble fraction of ethanolic extract of the dried fruits of Sorbus aucuparia (DC) four triterpenes were isolated and identified as α-amyrin, ursolic acid, methyl 2α-hydroxy ursolate, 2α-hydroxy ursolic acid in addition to β-sitosterol. The major component of ethyl acetate fraction was isolated and identified as rutin. Their structures were established by physical, chemical and spectral methods. This is the first report of isolation of ursolic acid and its derivatives from the plant.

INTRODUCTION

Sorbus aucuparia (DC) is grown in Europe and North Asia.[1] The false fruits are composed of the succulent part of receptacle enclosing mature ovary. It is ovoid, orange red and/or crimson in color.[2,3] The fruits have been reported to contain tannin, sorbitol, sorbic acid, sucrose, malic acid, vitamin C, hydroxy-diphenyl derivatives and lignan xyloside.[4] The fruit of *Sorbus aucuparia* (DC) was reported to have several folkloric uses in cough, catarrh and in diarrhea, where it was found to be active against gram +ve bacteria and protozoa.[5] Now, here we report the isolation and identification of the triterpenoidal content as well as rutin from the fruits of *Sorbus aucuparia* (DC).

EXPERIMENTAL

Plant material

The mature fruits were collected in May 1994 from Public gardens in Assiut University Club. It was identified and authenticated by Prof. Dr. A. Faid, Professor of Plant Taxonomy, Dept. of Botany, Faculty of Science, Assiut University. The fruits were oven-dried at 45°C, powdered and kept in well closed dark containers till use.

Extraction and isolation

The dried powdered fruits (2.5 kg) were extracted with 75% ethanol by maceration and percolation. The ethanolic extract was concentrated under reduced pressure to give a syrupy consistency (50 g), mixed with water and successively extracted with petroleum-ether, chloroform, ethyl acetate and n-butanol. TLC examination of chloroformic soluble fraction (10 g) revealed nearly 6 major spots on silica gel using $CHCl_3$:Methanol (95:5) and 30% H_2SO_4 as spraying reagent. The extract was subjected to silica gel column chromatography (400 g). Elution was performed with chloroform and then increasing the polarity gradiently with methanol. Fractions of 100 ml each were collected, similar fractions (TLC) were pooled together and

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subjected to crystallization where five compounds were isolated (**1-5**). Also, about 5 gm of ethyl acetate soluble fraction was chromatographed on silica gel. Elution was performed with $CHCl_3$ and increasing the polarity with methanol. The major compound eluted with $CHCl_3$-MeOH (8:2) was isolated as yellow amorphous material (compound **6**).

Compound 1: α-Amyrin

Obtained as white needles from methanol (m.p. 184-186°C). It gave violet color with Liebermann Burchards test.[6] This compound was identified as α-amyrin by direct comparison (m.p., m.m.p. and cochromatography with an authentic reference sample).

Compound 2: ß-Sitosterol

Needle-shaped crystals from absolute methanol (m.p. 135-137°C). Physical and spectral properties were found to be identical with those of the authentic ß-sitosterol (m.p., m.m.p. and cochromatography).

Compound 3: Ursolic acid

Fine-needles from methanol (m.p. 255-256°C), IR υ_{max}(KBr) cm^{-1} 3450, 2950, 1680, 1450, 1380; MS m/z: 456 $[M^+]$, 438 $[M-H_2O]^+$, 411 $[M-COOH]^+$, 393 $[M-H_2O-COOH]^+$, 248 (100%) and 208 (17.6%); ^{1}H-NMR ($CDCl_3$) δ 5.25 (1H, t), 3.65 (1H, m), 3.2 (1H, m), 2.2 (1H, d), 1.2-1.9 for methylenic protons, 1.1 (3H, s), 0.98 (3H, s), 0.95 (3H, d), 0.93 (3H, s), 0.87 (3H, d), 0.83 (3H, s), 0.78 (3H, s).

Compound 4: Methyl 2α-hydroxy ursolate

Needles from methanol (m.p. 213°C), IR υ_{max}(KBr) cm^{-1} 3335, 1725; MS m/z 486 $[M^+]$; ^{1}H-NMR ($CDCl_3$) δ 0.74 (3H, s), 0.82 (3H, s), 0.92 (6H, d), 0.99 (3H, s), 1.02 (3H, s), 1.07 (3H, s), 1.2 (3H, s), 2.9 (1H, d), 3.54 (3H, s), 3.62 (1H, d, J= 3 Hz), 3.72 (1H, m), 5.16 (1H, t).

Compound 5: 2α-Hydroxy ursolic acid

Fine needles from methanol (m.p. 298-300°C); IR υ_{max}(KBr) cm^{-1} 3500, 1995, 1612; MS m/z 472 $[M^+]$, 454 $[M-H_2O]^+$, 436 $[M-2H_2O]^+$. ^{1}H-NMR (CD_3Cl) δ 0.74 (3H, s), 0.82 (3H, s), 0.92 (6H, d), 0.99 (3H, s), 1.03 (3H, s), 1.08 (3H, s), 2.98 (1H, d), 3.62 (1H, d, J= 3 Hz), 3.72 (1H, m), 5.25 (1H, t).

Compound 6: Rutin

Yellow amorphous powder from methanol (m.p. 190-192°C). The UV data (Table 1) indicated that this compound is a flavonol glycoside containing ortho-hydroxy groups and free hydroxyl group at C-7; ^{1}H-NMR ($CDCl_3$) CH_3 rhamnose δ (1.0, d), protons (4.4, s), anomeric proton (5.3, d), 6.2 (H_2, d), 6.4 (H_6, d), 6.8 (H_5, d), 7.5 (H_8, dd), 7.6 (2H, d). ^{13}C-NMR C_2 (156.4), C_3 (133.3), C_4 (177.3), C_5 (161.2), C_6 (98.6), C_7 (164.1), C_8 (93.5), C_9 (156.5), C_{10} (104), $C_{1'}$ (121.2), $C_{2'}$ (115.2), $C_{3'}$ (144.7), $C_{4'}$ (148.4), $C_{5'}$ (116.2), $C_{6'}$ (121.5). Sugar glucose [C_1 (101.2), C_2 (74.0), C_3 (76.4), C_4 (70.0), C_5 (75.9), C_6 (67.0)]. Rhamnose [C_1 (100.7), C_2 (70.5), C_3 (70.3), C_4 (71.8), C_5 (68.2), C_6 (17.7)].

Table 1: UV spectral data of Rutin

Reagent / Band	MeOH	$AlCl_3$	$AlCl_3$ + HCl	NaOAc	NaOAc + H_3BO_3	NaOH
I	361 302 sh.	432+71 332	404+43 356	390+29 220	378+7	411+50
II	267 258 sh.	266+8	272+14 293 sh.	273+15	266+8	275+17

RESULTS AND DISCUSSION

α-Amyrin and ß-sitosterol were identified respectively "m.p., m.m.p and co-chromatography" with authentic samples. Chromatographic fractionation of the chloroformic extract yielded three triterpenoid compounds identified as ursolic acid, methyl 2α-hydroxy ursolate and 2α-hydroxy ursolic acid on the bases of physical, chemical and spectroscopic evidence (IR, NMR, MS).[7-9]

This is the first report on the presence of the above mentioned triterpenoid compounds in *Sorbus aucuparia* (DC). Rutin was identified from the inspection of the UV. data of the compound before and after hydrolysis and further confirmation was achieved by ^{1}H-NMR and ^{13}C-NMR which coinicide with those reported for rutin.[10,11]

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