Benzine Extract from *Sonchus asper*—A Rich Source of Pentacyclic Triterpenoids

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Pentacyclic triterpene alcohols (9.8%), their acetates (16.1%), and their long chain fatty acid esters (4.6%) represent 30.5% of the benzine extract from the herb of *Sonchus asper* Vill. (Compositae). The composition of triterpene alcohols was: α -amyrin (4%), β -amyrin (8%), germanicol (6%), ψ -taraxasterol (17%), taraxasterol (36%), lupeol (28%), and other unidentified components (1%); and that of triterpene acetates was: α -amyrin acetate (3%), β -amyrin acetate (8%), germanicol acetate (5%), ψ -taraxasterol acetate (19%), taraxasterol acetate (21%), and lupeol acetate (44%). Triterpene alcohol moiety of the long chain fatty acid esters was ψ -taraxasterol only. The results indicate that the benzine extract from *S. asper* Vill. can be used as a good source of pentacyclic triterpenoids.

1 Introduction

Sonchus asper Vill. is a annual herb of the family Compositae, commonly known as "Jan-gali Gobhi". It is pounded and applied to wounds and boils¹⁾. Considering its medicinal use and the fact that no work has so far been carried out on the chemical constituents, the present study was undertaken in an attempt to elucidate the compositional patterns of the benzine extract of this herb. This paper is concerned with the triterpenoid constituents.

2 Experimental

2.1 Materials

Sonchus asper, collected on the campus of Shri Varshney College, Aligarh (India), was air-dried, ground, and extracted in a Soxhlet extractor with benzine (bp 60~80°C) for 18 h. A yellow viscous oil (2.1%) was obtained after removal of solvent under reduced pressure.

The origin of the following authentic triter-

pene alcohols was described in the previous paper²⁾: α -amyrin (5 α -urs-12-en-3 β -ol), β -amyrin (5 α -olean-12-en-3 β -ol), germanicol (5 α -olean-18-en-3 β -ol), taraxasterol (5 α -taraxast-20[29]-en-3 β -ol), and ψ -taraxasterol (5 α -taraxast-20-en-3 β -ol). Lupeol (5 α -lup-20[29]-en-3 β -ol) was generously supplied from Dr. M. P. Khare as a gift.

2.2 General

Crystallizations were performed in acetone-methanol. Melting points determined on a heat block were uncorrected. Hydrolysis of fatty acid esters was performed by refluxing with alcoholic 1 N potassium hydroxide for 1 h, and acetylation of triterpene alcohols was accomplished with acetic anhydride and pyridine at room temp. over night.

TLC was carried out on Silica Gel G plates with carbon tetrachloride-ethyl acetate (95 : 5, vol/vol) as the developing solvent. The following R_f -values are illustrative: hydrocarbons 1.0, esters of higher fatty acids 0.9, triterpene

acetates 0.82, triterpene alcohols 0.51, and sterols 0.33. Preparative argentation TLC on plates coated with 0.5 mm layers of Silica Gel G impregnated with 20% silver nitrate was developed 5 times with methylene chloride-carbon tetrachloride (1 : 5, vol/vol). GLC was performed by a Shimadzu GC-4 CM instrument equipped with a flame ionization detector on Poly I-110 (column 270°C, injection 285°C, carrier gas 80 ml/min, split ratio 50 : 1, scavenge gas 80 ml/min, retention time of cholesterol acetate ca. 20 min) and on OV-17 (column 260°C, injection 280°C, carrier gas 60 ml/min, split ratio 50 : 1, scavenge gas 80 ml/min, retention time of cholesterol acetate ca. 18 min) in the form of SCOT glass capillary columns (30 m × 0.3 mm i. d., Wako Pure Chemical Industries Ltd., Osaka). The relative retention time (RRT) was given relative to cholesterol acetate. Since the component peaks with long retention time appeared with "leading" on OV-17, the retention time of GLC peaks was defined here on both Poly I-110 and OV-17 columns at the starting point of the elution peak appearance instead of at the usual top edge of the peak. Quantitative evaluation of each triterpene component was carried out based on the argentation TLC data and on the GLC data on Poly I-110.

IR spectra were recorded on a Perkin-Elmer spectrometer using KBr discs. Nuclear magnetic resonance (NMR) spectra were recorded either on a Varian spectrometer A-60 D or on a JNM-FX 100 (Japan Electron Optics Laboratory Co., Tokyo), in deuteriochloroform using tetramethylsilane as the internal standard. Chemical shifts are expressed in δ -scales (ppm). Mass spectra (70 eV) were taken with a Shimadzu LKB-9000 gas chromatograph-mass spectrometer (2 m × 3 mm i. d. glass column packed with a 2% OV-17 on Gas Chrom-Q).

3 Results

The benzine extract was chromatographed by column chromatography on activated alumina. The column was developed with hexane and benzine-benzene. The eluate was monitored periodically by the TLC and separated into the following five fractions: pentacyclic triterpene alcohols (9.8% on the

extract basis), their acetates (16.1%) and their higher fatty acid esters (4.6%), and hydrocarbons and sterols.

3.1 Higher Fatty Acid Esters of Triterpene Alcohols

The alcohol obtained after hydrolysis of the fatty acid ester fraction was acetylated. The acetate was separated on argentation TLC plate into three zones, aliphatic alcohol acetates (fraction 1), sterol acetates (fraction 2), and ψ -taraxasterol acetate (fraction 3); the fractions were numbered in the order of polarity, beginning with the least polarity. Identification of ψ -taraxasterol was performed by co-TLC and GLC with an authentic specimen as the acetate.

3.2 Triterpene Acetates

The fraction of triterpene acetates exhibited prominent IR bands at 1730, 1248 cm^{-1} (acetate), 1640 cm^{-1} (C=C), 1366, 1388 cm^{-1} (geminal dimethyl group). NMR spectrum of the acetate gave the signals at δ 0.75, 0.81, 0.86, 0.96, 1.05, 1.27, 1.41 and 1.46; singlet at δ 2.03 ($-\text{OCOCH}_3$) and multiplet at δ 4.29~4.6 ($>\text{CHOAc}$), 4.59~4.69 ($>\text{C}=\text{CH}_2$), and 4.76~4.92 ($>\text{C}=\text{CH}-$)³⁾. This fraction was fractionated by preparative argentation TLC to give eight principal zones, i to viii, in the order of decreasing R_f -values.

(i) α -Amyrin acetate: The compound separated from the first zone was crystallized as fine plates (3%), having mp 221~223°C (lit.⁴⁾ mp 226°C), R_f -value 0.61, RRT 1.98 (Poly I-110), 1.84 (OV-17). The mass spectrum of this acetate showed M^+ at m/e 468 (18%) and other ions at m/e 453 (4.4), 408 (3.3), 249 (4.2), 218 (100), 203 (16), 189 (12). The strong peak at m/e 218 is characteristic for the α - and β -amyrin series. The RRT and the basic fragmentation pattern⁵⁾ of this compound confirmed it to be α -amyrin acetate.

(ii) β -Amyrin acetate: The second zone gave fine prisms after crystallization (8%), having mp 241~244°C (lit.⁴⁾ mp 241°C), R_f -value 0.57, RRT 1.68 (Poly I-110), 1.64 (OV-17). The mass spectrum of the acetate showed M^+ at m/e 468 (15%) and other ions at m/e 453 (5.0), 408 (2.2), 249 (2.4), 218 (100), 203 (24), 189 (8.0). This compound was

confirmed as β -amyrin acetate on the basis of mass fragmentation⁵³ (strong peak at m/e 218) and RRT data.

(iii): This zone consisted of a mixture of several unidentified compounds (<0.5%).

(iv); **Germanicol acetate**: The compound separated from the zone iv was crystallized as fine plates (5%), having mp 279~281°C (lit.⁶³ mp 281~282°C), R_f -value 0.37 and RRT 1.68 (Poly I-110), 1.64 (OV-17). The mass spectrum of the triterpene acetate showed M^+ at m/e 468 (17.5%) and other ions at m/e 453 (18.0), 408 (8.2), 393 (9.0), 218 (23.5), 204 (100), 189 (95.0), 177 (70.0), which is identical with that of germanicol acetate reported in the literature and the spectrum was fully agreeable to that compound⁶³⁻⁶⁵. The NMR spectrum was also in accordance with that of germanicol acetate reported in the literature^{77, 93}: 3β -Ac δ 2.05, C-3 (CH-O) 4.47, C-23, C-24 ($2\times$ CH₃) 0.84, C-25 (CH₃) 0.90, C-26 (CH₃) 1.07, C-27 (CH₃) 0.73, C-28 (CH₃) 1.01, C-29 (CH₃) 0.94, C-30 (CH₃) 0.94, C-19 (olefin) 4.85. Assignment of each signal in the spectrum was carried out by the experiment with a lanthanide shift reagent, *tris* (dipivaloylmethanato) europium, in a similar manner as described by Shingu *et al.*¹⁰⁰ Thus on the basis of mass fragmentation and NMR spectrum this compound was confirmed as germanicol acetate. The chromatographic and spectral data of authentic germanicol acetate supported the identification.

(v) **Ψ -Taraxasterol acetate**: The compound separated from the zone v was crystallized as fine needles (19%), having mp 236~238°C (lit.¹¹³ mp 235°C), R_f -value 0.33 and RRT 2.66 (Poly I-110), 2.41 (OV-17). The mass spectrum of the acetate showed M^+ at m/e 468 (12.9%) and other ions at m/e 453 (11.8), 408 (9.6), 218 (67.8), 203 (35.8), 189 (100). This compound was confirmed as Ψ -taraxasterol acetate on the basis of mass fragmentation⁵³ and RRT data.

(vi) **Taraxasterol acetate**: It was crystallized as fine plates (21%), having mp 249~251°C (lit.¹¹³ mp 251~2°C), R_f -value 0.29 and RRT 2.71 (Poly I-110), 2.51 (OV-17). The mass spectrum of the acetate gave M^+ at m/e 468 (16.4%) and other peaks at m/e 453 (7.3),

408 (13.3), 393 (8.7), 218 (4.5), 203 (31.7), 189 (100). The RRT and the fragmentation pattern of the triterpene acetate were identical with those of authentic taraxasterol acetate¹²³. Hence this triterpene acetate was recognized as taraxasterol acetate.

(vii): This zone (<0.5%) was a mixture of taraxasterol acetate and lupeol acetate.

(viii) **Lupeol acetate**: This compound was crystallized as fine needles (44%), having mp 219~220°C (lit.⁴³ mp 220°C), R_f -value 0.10 and RRT 1.91 (Poly I-110), 1.92 (OV-17). Mass spectrum of the acetate gave M^+ at m/e 468 (11.4%) with other principal ions at m/e 453 (5.8), 408 (15.8), 393 (8.2), 218 (15.4), 203 (19.8) and 189 (100). The RRT and basic fragmentation patterns agreed with those of authentic lupeol acetate⁵³. Consequently the triterpene acetate was recognized as lupeol acetate.

3.3 Triterpene Alcohols

The triterpene alcohol fraction after acetylation was separated into two fractions by column chromatography on silica gel impregnated with 20% silver nitrate. The 2nd major fraction was found to be a mixture of six triterpene alcohols, identified as the acetates on the basis of co-TLC and GLC as α -amyrin (4%), β -amyrin (8%), germanicol (6%), Ψ -taraxasterol (17%), taraxasterol (36%) and lupeol (28%), accompanied with other minor unidentified components (1%).

4 Discussion

Pentacyclic triterpenoids have been found as the minor components in many seed oils^{23, 123} and often represent the greater part of the unsaponifiable portion of these oils. They are usually associated with hydrocarbons, sterols and other triterpene alcohols, especially with cycloartenol and 24-methylenecycloartanol. A few oils of plant origin, however, have been reported to contain a large amount of pentacyclic triterpenoids: the ethanol extract containing as high as about 80% of pentacyclic triterpenoids from the latex exuded from the bark of *Achras sapota*¹⁴³, the petroleum ether extract containing about 40% of pentacyclic triterpenoids from the fruit (seed and pericarp) of two *Jurinea* species¹⁵³, and the petroleum

ether extract containing about 50% of pentacyclic triterpenoids from the seed and pericarp of *Carduus nigrescens*⁵⁾.

Our finding indicates that pentacyclic triterpenoids occur in the extract of *S. asper* as the acetate, esters of higher fatty acids as well as in the form of triterpene alcohols, along with hydrocarbons, esters of aliphatic alcohols, and sterols. The total yield of all the triterpenoids has been found to be 30.5% of the benzene extract of the plant and indicates that the extract of *S. asper* can be used as a good source of pentacyclic triterpenoids.

Acknowledgments

The authors are grateful to Dr. S. C. Gupta and Dr. W. Rahman (Aligarh) for facilities. Thanks are also due to Dr. S. Singh (Lucknow) for IR and NMR spectra, and to U.G.C., Delhi for J.R.F. to R. Gupta. Prof. Y. Toyama contributed valuable comments and advices.

(Received May 15, 1979)

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五環性トリテルペノイド成分に富む

おにのげしのベンジン抽出油

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おにのげし(きく科)のベンジン抽出物は五環性トリテルペノイド成分に富み、遊離の五環性トリテルペンアルコール(9.8%), それらのアセタート(16.1%)及び長鎖脂肪酸エステル(4.6%)で、その和は全抽出物の30.5%をも占めていた。遊離トリテルペンアルコール混合物の組成は α -アミリン(4%), β -アミリン(8%), ゲルマニコール(6%), ψ -タラクスステロール(17%), タラクスステロール(36%), ルペオール(28%)及び未同定成分(1%)であり、トリテルペンアセタート混合物の組成は α -アミリンアセタート(3%), β -アミリンアセタート(8%), ゲルマニコールアセタート(5%), ψ -タラクスステロールアセタート(19%), タラクスステロールアセタート(21%)及びルペオールアセタート(44%)であった。一方、長鎖脂肪酸エステルより得られたトリテルペン成分としては ψ -タラクスステロールのみが検出された。以上の結果は、おにのげし油が五環性トリテルペノイドを得る優れた原料であることを示すものである。