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VERGATIC ACID, A NEW PENTACYCLIC TRITERPENE FROM *SALVIA VIRGATA*

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Key Word Index—*Salvia virgata* Jacq.; Labiatae; a new pentacyclic triterpene; virgatic acid.

Abstract—A new pentacyclic triterpene acid was isolated from the arial parts of *Salvia virgata* and its constitution was established as 3β-hydroxy-1-oxo-olean-12-en-28-oic acid and named virgatic acid.

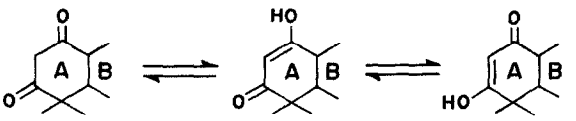
INTRODUCTION

Pentacyclic triterpenoids are common in *Salvia* species. Ursolic and oleanolic acids were isolated from *S. triloba* [1–4], *S. officinalis* [3,5–11], *S. calycina* [3], *S. glutinosa* [12], *S. pratensis* and *S. sclarea* [13]. Micromeric acid was obtained from *S. horminum* [14], α- and β-amyrin were found in *S. apiana* [15] and *S. officinalis* [16], anagadiol was isolated from *S. broussuneti* [17]. This paper describes a new triterpene acid which was obtained from the chloroform extracts of *S. virgata*. The compound has been named virgatic acid and its structure has been established.

RESULTS

The petrol extract of the arial parts of *S. virgata* yielded a small amount of a new triterpene together with flavonoids [18]. The chloroform extract of the mark on column chromatography gave the same compound in a high yield. This was named virgatic acid, $C_{30}H_{46}O_4$. IR indicated a hydroxyl (3450 cm^{-1}), a six membered ring carbonyl (1722 cm^{-1}) and a carboxyl (1697 cm^{-1}). The NMR spectrum showed methyl singlets at δ 0·89, 0·96, 1·03 and 1·25 corresponding to seven methyl groups and a hydroxyl proton at 1·72 (D_2O exchange). A well divided triplet at 3·42 showed a hydrogen next to hydroxyl group and indicated the presence of $-\underline{CH}(OH)CH_2-$, a one hydrogen triplet at 5·46 suggested a Δ^{12} double bond. Acetylation of the compound gave the corresponding monoacetate.

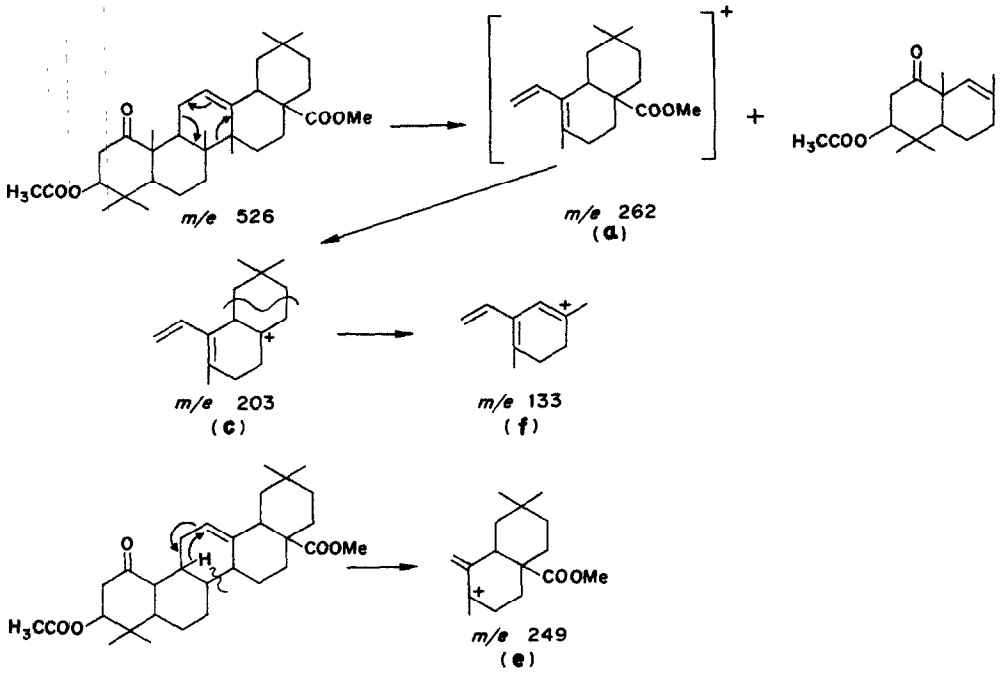
The above mentioned data and the MS of the methyl ester acetate of virgatic acid suggested a derivative of an olean-12-en-oic acid. The MS (see Scheme 1) showed diagnostically important peaks at 526 (M^+), 262 (**a**), 203 (**c**), 133 (**f**) and 249 (**e**). The peaks at *m/e* 262 (**a**) and 203 (**c**) showed that the D and E rings were the same as in oleanolic acid. The peak at *m/e* 203 is especially characteristic for 28-oic acids and seems to have arisen by the loss of the –COOMe group from fragment (**a**). The base peaks were at *m/e* 262 and 203. A Retro–Diels–Alder fragmentation pattern is characteristic for Δ^{12} pentacyclic triterpenes [19]. The small peak at *m/e* 249 (**e**), comprising of the D and E rings arose by a fragmentation involving one hydrogen transfer and cleavage of an allylically activated bond.



The MS fragmentation pattern restricted the allocation of the hydroxyl and carbonyl groups to rings A and B. The position of the acid group was confirmed by the fact that pentacyclic triterpenic acid methyl esters give in their NMR spectra the carboxy methyl peak at δ 3·59 or less, if the acid group is at C-28. In the cases where the acid group is at another location the NMR peak appears between δ 3·59 and 3·62. Virgatic acid gave this peak at δ 3·59 in its methyl ester acetate and δ 3·56 in its methyl ester derivative [20]. The Δ^{12} position of the double bond and the C-17 position of the carboxyl group were supported by the formation of a lactone with bromine.

There are several publications describing the effects of hydroxyl and/or oxo groups on the shifts of methyl peaks in NMR spectra [21–24]. The shifts in the methyl peaks of virgatic acid and its acetyl derivative were calculated according to the above mentioned papers and they verified the C-3β position for the hydroxyl group. The 3β-hydroxyl is in accord with biogenetic considerations and is also in agreement with the observed NMR triplet at δ 3·42. In order to further confirm the 3β-hydroxyl and C-28 carboxyl groups the Huang–Minlon reduction was performed [25]. The IR spectrum, the R_f values and mmp of the resulting compound showed that it was identical to authentic oleanolic acid.

$NaBH_4$ reduction of the acid and Jones oxidation of the resulting diol caused an enolization (Scheme 2) to give a compound with a λ_{max} at 254 nm indicating that the carbonyl group should be at the C-1 position. The formation of the enol ruled out the possibility of ring B having the carbonyl group. The alternative position of the carbonyl group could be at C-2, but in this case the λ_{max} of the enol should be around 270 nm. Also in



the NMR spectrum the C-3 proton would then be a singlet instead of the observed triplet. The C-1 position of the carbonyl was also confirmed by the failure to form an isopropylidene derivative of the diol and the absence of oxidation with periodate.

$LiAlH_4$ reduction of the compound yielded a triol, the carbonyl and carboxyl peaks disappeared from the IR spectrum. NMR gave a *dd* at δ 3·85 ($-C\underline{H}_2OH$), formed by the reduction of the carboxyl group. Acetylation of this compound gave the corresponding triacetate.

A survey of the literature [26] showed that momordic acid was assigned the same structure as that now given for virgatic acid. However, personal correspondence with Prof. T. Murakami revealed that momordic acid was not a new compound, it was later found to be gibsogenin. Therefore the triterpenic acid obtained from *S. virgata* is a new compound for which we propose the name virgatic acid and its structure is 3β-hydroxy-1-oxo-olean-12-en-28-oic acid.

EXPERIMENTAL

All mps were taken with a Reichert microscope melting point instrument and are uncorrected.

Extraction and isolation of virgatic acid. The plant was collected at Aydos (near Istanbul) in June 1972 and identified as *Salvia virgata* Jacq. by Prof. Dr. A. Baytop (Istanbul), a voucher sample is deposited in the herbarium of the Faculty of Pharmacy, University of Istanbul. Dried arial parts of *S. virgata* (4 kg) were extracted first with petrol, then with $CHCl_3$ and the mark was discarded. Evaporation of the $CHCl_3$ extract gave 76 g of a light green residue and 20 g of this was chromatographed over Si gel (0·05–0·2 mm). The $CHCl_3$ and $CHCl_3$–EtOH (4:1) eluates yielded a single compound (2·3 g), EtOAc crystallization gave a white crystalline compound (600 mg). TLC, argentation TLC, as well as GLC of its methyl ester showed that this was a single compound. The acid gave a positive Liebermann–Burchard test, mp 226–228°. (Found: C, 76·55; H, 9·85. $C_{30}H_{46}O_4$ requires: C, 76·59; H, 9·76%.) IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1722 (carbonyl), 1697 (carboxyl), 1060 (C–O), 1375 and 1385 (gem dimethyl). NMR (C_5D_5N): described in the text. MS *m/e* 470 (M^+), 249 (D and E rings), 204 (249–COOH).

Monoacetate of virgatic acid. The acid (80 mg) in 2 ml C_5H_5N was treated with Ac_2O (1 ml) for 24 hr at room temp., the monoacetate was crystallized from EtOAc as white small crystals, mp 168°. (Found: C, 74·70; H, 9·70. $C_{32}H_{48}O_5$ requires: C, 75·00; H, 9·37%.) IR ν_{max}^{KBr} cm^{-1}: 3450 (OH of the acid), 1740 (acetyl), 1700 (carbonyl and carboxyl), 1250 (acetoxy methyl). NMR (100 MHz, $CDCl_3$): δ 0·79, 0·89, 0·98, 1·10 and 1·28 (seven tertiary methyl singlets), 2·06 (3H, *s*, acetyl), 4·50 (1H, *t*, $-C\underline{H}$(OCOMe), 5·24 (1H, *t*, $-C{=}C\underline{H}-$).

Methyl ester of virgatic acid. The compound (50 mg) dissolved in MeOH and methylated with excess CH_2N_2 in Et_2O at 5°, crystallized from $CHCl_3$–MeOH (1:1), mp 148°. (Found: C, 76·84; H, 10·05. $C_{31}H_{48}O_4$ requires: C, 76·86; H, 9·92%.) IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1725 (ester and ring carbonyl), 1385 (gem dimethyl), 1050 (C–O). NMR (C_5D_5N): δ 0·75, 0·78, 0·89, 0·96, 1·07, 1·25 (methyl singlets), 3·56 (3H, *s*, COOMe), 5·20 (1H, *t*, $-C{=}C\underline{H}-$), 3·40 (1H, *t*, $-C\underline{H}OH$).

Methyl ester acetate. Virgatic acid acetate (100 mg) was methylated with CH_2N_2, crystallized from $CHCl_3$–MeOH (1:1), mp 137°. (Found: C, 75·52; H, 9·73. $C_{33}H_{50}O_5$ requires: C, 75·28; H, 9·51%.) IR ν_{max}^{KBr} cm^{-1}: 1735 (acetyl and ester carbonyl), 1720 (six member ring carbonyl), 1250 (acetoxy methyl). NMR ($CDCl_3$): δ 0·76, 0·86, 0·86, 0·94, 1·08, 1·25 (methyl singlets) 2·06 (3H, *s*, acetyl), 3·59 (3H, *s*, COOMe), 4·50 (1H, *t*, $-C\underline{H}$(OCOMe), 5·20 (1H, *t*, $-C{=}C\underline{H}-$).

Virgatic acid bromo-γ-lactone. A soln of virgatic acid (30 mg) in MeOH (2 ml) was treated with Br_2 (10 mg) in MeOH (1 ml). After 1 hr the soln was cooled to give crystals, mp 165°. (Found: C, 65·68; H, 8·52. $C_{30}H_{45}O_4Br$ requires: C, 65·39; H, 8·37%.) IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1760 (lactone) 1720 (ring carbonyl). NMR: no olefinic proton.

$NaBH_4$ reduction of virgatic acid. Virgatic acid (20 mg) dissolved in 2 ml MeOH was refluxed with 50 mg $NaBH_4$ for 3 hr. After cooling 3 ml of 3 N HCl was added and the mixture left for 24 hr before extraction with Et_2O. The Et_2O soluble part was purified on a small Si gel column, the compound obtained from the $CHCl_3$ and EtOH eluates was pure but not crystalline. (Found: C, 76·86; H, 10·49. $C_{30}H_{48}O_4$ requires: C, 76·27; H, 10·17%.) IR ν_{max}^{KBr} cm^{-1}: 3450 (hydroxyl),

1700 (carboxyl), 1030, 1045 (C–O). NMR (C_5D_5N): δ 0·85, 0·92, 1·08, 1·15, 1·22 (methyl singlets, 3·42 (1H, *t*, C-3), 3·62 (1H, *t*, C-1), 5·46 (1H, *t*, –C=C<u>H</u>–).

Jones oxidation of the diol. The diol (15 mg) obtained from $NaBH_4$ reduction was dissolved in 3 ml Me_2CO and 1 ml Jones reagent (CrO_3–AcOH) was added at room temp. After shaking 5 min it was diluted with H_2O and extracted with $CHCl_3$. After removal of $CHCl_3$ the residue was purified by column chromatography over Si gel. $CHCl_3$–EtOH eluates yielded the enol as a gummy material. Negative ferric reaction: UV λ_{max} 254 nm, alkali addition caused a bathochromic shift to 286 nm. IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1715 (carboxyl and carbonyl). NMR (C_5D_5N): δ 0·86, 0·92, 1·08, 1·15, 1·25 (methyl singlets), 5·42 (1H, *t*, C-12), 6·32 (1H, *s*, C-2).

Huang–Minlon reduction of virgatic acid. A mixture of 100 mg virgatic acid, 50 mg NaOH, 2 ml diethylene glycol and 0·5 ml 85% hydrazine hydrate was refluxed for 3 hr and then an aq. soln was eliminated by removing the condenser until the temp of the soln reached 195–200° and continuing refluxing for 2 hr. The reaction mixture was made acidic by the addition of 6 N HCl and extracted with Et_2O. The residue obtained from the evaporation of the Et_2O crystallized from $CHCl_3$–MeOH (1:1), mp 305°. (Found: C, 79·05; H, 10·79. $C_{30}H_{48}O_3$ requires: C, 78·72; H, 10·52%.) Mixed mp, IR spectra and R_f value comparisons with authentic oleanolic acid established the identity of the product.

$LiAlH_4$ reduction of virgatic acid. Virgatic acid (250 mg) was dissolved in 20 ml THF and 750 mg $LiAlH_4$ in 35 ml Et_2O was slowly added. After refluxing for 44 hr excess $LiAlH_4$ was destroyed with aq. Et_2O, the mixture filtered and evaporated to dryness. The triol was crystallized from MeOH, mp 212–214°. (Found: C, 78·55; H, 10·99. $C_{30}H_{50}O_3$ requires: C, 78·60; H, 10·87%.) IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1470, 1390, 1385 (C–H), 1050, 1030, 1010 (C–O). NMR (C_5D_5N): δ 0·96, 1·02, 1·10, 1·26 (methyl singlets), 3·50 (2H, *t*, C-1 and C-3), 3·85 (2H, *dd*, –C<u>H</u>$_2$OH), 1·78 (1H, *br*, *s*, OH, D_2O), 4·95 (1H, *br*, *s*, OH, D_2O), 5·22 (1H, *t*, –C=C<u>H</u>R-), 5·77 (1H, *br*, *s*, OH, D_2O).

Acetyl derivative of the triol. The triol (50 mg) was acetylated in the usual way, mp 160°. (Found: C, 74·05; H, 9·85. $C_{36}H_{56}O_6$ requires: C, 73·97; H, 9·59%.) IR ν_{max}^{KBr} cm^{-1}: 2950, 2880 (C–H), 1735, 1730 (acetyl), 1470, 1390, 1385 (C–H), 1250 (acetoxy methyl). NMR ($CDCl_3$): δ 0·85, 0·95, 1·08, 1·16, 1·22 (methyl singlets), 2·01 (9H, *s*, acetyl), 3·62 (2H, *t*), 3·96 (2H, *t*), 4·46 (1H, *t*), 5·10 (1H, *m*), 5·46 (1H, *t*).

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