A New Flavone from *Salvia triloba L.f* (*Labiatae*)

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Salvigenin was isolated from the air-dried leaves and stems of *S. triloba*. Seventy percent aqueous ethanolic extract of the plant yields ursolic acid, oleonolic acid, β-sitosterol, carnosol, and the above-mentioned new flavone, when separated on a silicilic acid–diatomaceous earth (3:1) column. Methylation of pectolinarigenin, C, H, and methoxy group determination, alcoholic KOH degradation, and UV, IR, and NMR methods led to the establishment of the following structure for salvigenin: 5-OH-6,7,4′-OCH_3-flavone or pectolinarigenin-7-methyl ether.

Salvia triloba L.f (*Labiatae*) which is a shrub 0.5–1.5 M. tall, is distributed from the Marmara sea to the Mediterranean. The plant is also known as mountain apple in Turkey.

A survey in literature showed that various salvia species such as *S. officinalis* (1–12), *S. braclyodon* (13), *S. pratensis* and *S. sclerae* (14), *S. miltiorrhiza* (15), *S. spinoza* (16), *S. splendens* (17), *S. glutinoza* (18) have been studied and ursolic acid I and II, oleolonic acid, sclareol, carnosol, tanshinone I, II, III, β-sitosterol, thujone, carene, azulene, α and β pinene, camphor, borneol, bornyl acetate, pentagalactosido sucrose, and acetyl anthocyans have been isolated and identified.

However, there are only a few papers on *S. triloba*. In 1954 Leiner (19) studied the ether extracts of *S. triloba* in this school and obtained ursolic acid I and II, oleonolic acid, a paraffin, a polyterpene and an oily compound, a flavone aglicon, a flavone glycoside, and a bitter substance. Ursolic acid I and II and oleonolic acid were obtained from the *S. triloba* in Greece (20). Brieskorn (21) found the same acids in *S. triloba*.

EXPERIMENTAL

The plant was collected from Marmara island, Marmara sea, Turkey, in May 1967. Five-hundred grams of the air-dried leaves and stems were pulverized and extracted with 3 L. of 70% aqueous alcohol. Alcoholic extract was evaporated under a vacuum and 20 Gm. of a green precipitate (I) was separated. The brown aqueous part upon evaporation to dryness yielded 100 Gm. of a residue (II).

Fifteen grams of I was chromatographed on a 3 × 50 cm. column, using silicilic acid–diatomaceous earth (3:1). Fifty fractions, 100 ml. of each, were collected using the following solvents:

Solvents for Collecting Fractions	Number of the fractions
Petroleum ether	1–2
Petroleum ether–benzene (9:1)	3–4
Petroleum ether–benzene (1:1)	5–6
Benzene	7–11
Benzene–chloroform (9:1)	12–13
Benzene–chloroform (1:1)	14–15
Chloroform	16–17
Chloroform–ether (9:1)	18–25
Chloroform–ether (1:1)	26–27
Ether	27–40
Alcohol	40–50

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Each fraction was checked on thin layer and those which gave the same spots were combined, thus 1–6, 7–8, 9–11, 12–18, 19–20, 21–23, 23–50 yielded seven fractions.

Fractions 1–8, being the essential oils of the plant, were not studied; fractions 23–50 were left aside together with the residue II for future studies.

Fractions 9–11 showed two distinct and five minor spots on thin layer; upon crystallization from boiling alcohol 70 mg. of a white crystalline compound was obtained. Thin layer, mixed m.p.'s, and IR curve comparison showed that this compound was β-sitosterol. The second compound was not obtained in the crystalline form.

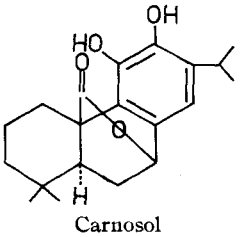
Fractions 12–18 when checked on thin layer showed one major and four minor spots; upon evaporation of the solvent these fractions yielded white needle-like crystals. Alcohol recrystallization yielded 1 Gm. of a compound; m.p. 286–290°, optical rotation $[\alpha]_D^{20}$ = +65 (in pyridin). Mixed m.p.'s and IR curve comparison as well as the optical rotation proved that this was ursolic acid.

Fractions 19 and 20 yielded two compounds, one of which was carnosol, the other was an unknown compound.

Fractions 21–23 yielded white crystals; alcohol recrystallization gave 250 mg. of a white crystalline material, m.p. 305°, optical rotation $[\alpha]_D^{20}$ = +83 (in methanol). Mixed m.p.'s and IR curve comparison as well as the optical rotation proved that this was oleonolic acid.

ISOLATION OF CARNOSOL

Fractions 19 and 20 when left aside yielded short, white, stick-like crystals (590 mg.) upon recrystallization from alcohol, the yield was 412 mg. Under the light, the crystals developed a blue-violet tinge. Melting point was 235–237°, UV spectra were taken using a Beckman DB recording instrument, $\lambda_{max.}$ 226 mμ (ε 12,100) and 288 mμ (ε 3,120) in ethanol. IR spectra (KBr) (Beckman IR-8) 3,570 $cm.^{-1}$ (intramolecular hydrogen bond), 3,330 $cm.^{-1}$ (OH groups), 2,980, 2,890 $cm.^{-1}$ (methyl groups), 1,720 $cm.^{-1}$ (lacton group), 1,580, 1,470, 1,450 $cm.^{-1}$ (aromatic ring), 1,380, 1,170 $cm.^{-1}$ (isopropyl group). NMR spectra (C_5D_5N) (TMS as internal



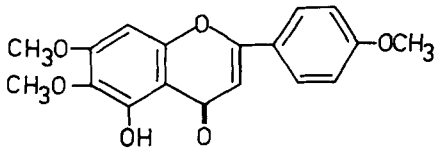
Carnosol

standard) (Varian Associates M-60) 0.8–1.05 p.p.m. (methyl groups in isopropyl chain), 1.3–1.5 p.p.m. (methyl groups in the nucleus), 6.93 p.p.m. (proton in the benzene ring), 5.62 p.p.m. (multiplet) (proton in the side chain), 1.65–4 p.p.m. (δ) (10 protons in the hydrogenated nucleus), and deuterium exchange show clearly the two OH groups.

Anal.—Calcd. for $C_{20}H_{26}O_4$: C, 72.72; H, 7.87. Found: C, 72.16; H, 7.87.

Infrared, NMR spectra, and analytical calculations suggested that this compound could be carnosol. Mixed m.p.'s and the IR curve comparison with the carnosol sample proved that these two compounds were identical.

Isolation and Identification of Salvigenin—The mother liquid of carnosol yielded green crystals; upon recrystallization from ethanol straw yellow-colored needles were obtained (70 mg.); m.p. 188°. UV maxima were 336 mμ (ϵ 31,000) and 280 mμ (ϵ 23,500). IR spectra suggested that this compound could be a flavone with a 5-OH group; 3,050 cm.$^{-1}$ (bonded OH), no free OH bands, 1,650 cm.$^{-1}$ (carbonyl group), 825 cm.$^{-1}$ (4′-OCH_3 group), 705 cm.$^{-1}$ (5-OH flavone). NMR spectra showed three methoxy groups at 4.0 p.p.m., 7.93, and 7.68 p.p.m. (aromatic H_2', H_6'); 7.1 and 6.93 (aromatic H_3', H_5'); 6.55 and 6.6 (aromatic H_8, H_3), and 11.6 p.p.m. (δ) (5-OH).



Salvigenin

Anal.—Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.87; —OCH_3, 28.36. Found: C, 65.72; H, 5.1; —OCH_3, 26.96

Methylation of Pectolinarigenin—Ten milligrams of pectolinarigenin was dissolved in acetone and methylated with diazomethane in ether according to Merz (22). The reaction mixture was evaporated and the yellow residue was crystallized from ethyl acetate. A mixed m.p. of pectolinarigenin-7-methyl ether with salvigenin and the IR curve comparison showed that they were identical.

Hydrolysis of Salvigenin—Hydrolysis of salvigenin with 0.1 *N* H_2SO_4, then with more concentrated acids did not yield any sugar residue.

Degradation of Salvigenin—Twenty milligrams of salvigenin was refluxed with alcoholic KOH for about 5 hr. The acidic and the phenolic parts of the mixture were separated according to a previous paper (23). 4,5-Dimethoxy resorcinol and *p*-methoxy benzoic acid were found by thin-layer comparison.

SUMMARY

A new flavone was isolated from the leaves and the stems of *Salvia triloba L.f*, and its structure was found by using UV, IR, and NMR methods, as well as the alkaline degradation and the partial synthesis of the compound from pectolinarigenin. Carnosol which was found in *Salvia officinalis* was also found in this plant and identified as well as the following compounds: ursolic acid, oleonolic acid, and β-sitosterol utilizing thin-layer column chromatography, UV, IR, and when necessary, NMR techniques.

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Keyphrases

Salvia triloba—phytochemical investigation
Salvigenin—new flavone, isolated
Column chromatography—separation
TLC—identity
Optical rotation—identity
NMR spectrometry—identity
IR spectrophotometry—structure
UV spectrophotometry—structure