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ISOLATION, STRUCTURE AND SYNTHESIS OF SCAPOSIN, A NEW FLAVONE FROM *HYMENOXYS SCAPOSA* DC. (COMPOSITAE)

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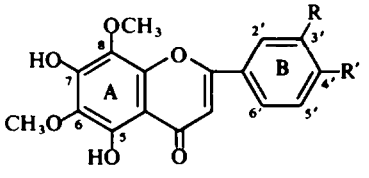
Abstract—A new flavone, scaposin, has been isolated from *Hymenoxys scaposa* (Compositae) and its structure established as 5,5',7-trihydroxy-3',4',6,8-tetramethoxyflavone by synthesis. Scaposin is the most highly oxygenated naturally occurring flavone known.

INTRODUCTION

RECENTLY, several members (**1–6**) of a rare group of fully oxygenated A-ring flavones having the 5,7-dihydroxy-6,8-dimethoxy substitution pattern have been described.[1–5] These compounds have 4'- or 3',4'-substitution in the B-ring. This paper describes the isolation, structure proof and synthesis of a new member of the series; it is the first



1, Demethoxysudachitin:[1]	R = H, R' = OH
2, Nevadensin:[2]	R = H, R' = OMe
3, Sudachitin:[1]	R = OMe, R' = OH
4, Acerosin:[3]	R = OH, R' = OMe
5, Lucidin:[4]	R, R' = O—CH_2—O
6, Hymenoxin:[5]	R = R' = OMe

with the 3',4',5'-oxygenation pattern in the B-ring, and thus represents the most highly oxygenated flavone yet reported from Nature.

Isolation and physical properties of scaposin

Chromatography of the crude methylene chloride extract of *Hymenoxys scaposa* (Compositae) leaves yielded demethoxysudachitin (**1**), hymenoxin[5] (**6**) and another yellow substance, $C_{19}H_{18}O_9$, m.p. 210–212°, which we named scaposin. The latter compound exhibited UV maxima in methanol at 330 and 281 mμ and gave shifts with diagnostic reagents (NaOMe, $AlCl_3$ and NaOAc) which suggested a close structural relationship with hymenoxin (**6**). The PMR spectrum (in DMSO-d_6) of scaposin indicated the presence of four OMe groups (7·94, 7·91, 7·83 and 7·82 ppm, δ-scale), three flavone-nucleus protons (one proton singlets at 7·25, 7·20 and 6·90 ppm) and a C-5 hydrogen-bonded OH group (12·71 ppm). The two singlets at 7·25 and 7·20 ppm

suggested that the molecule had an unsymmetrical 3',4',5'-trisubstituted B-ring and the singlet at 6·90 ppm could be attributed to either a C-3, C-6 or a C-8 proton. The PMR spectrum of the fully trimethylsilylated compound[6] (in CCl_4) displayed singlets at 7·07 (two protons) and 6·37 ppm. When the derivative was allowed to stand open to the atmosphere for 30 min and the PMR spectrum analysis was repeated, the singlet at 6·37 ppm had shifted downfield to 6·48 ppm and a new singlet appeared at 12·40 ppm. The latter signals indicated that hydrolysis of the C-5 trimethylsilyl ether had occurred. The downfield shift observed for the 6·37 ppm signal on de-trimethylsilylation of the C-5 OH group is typical for the H-3 signal in flavones (the H-8 signal characteristically moves upfield and the H-6 signal is unaffected).[6] On the basis of the above spectral data, scaposin could therefore be assigned a trihydroxy-tetramethoxyflavone structure which contained a OH group at C-5 and the other substituents (two OH and four OMe groups) at the 3',4',5',6,7 and 8-positions. Addition of fused sodium acetate to a methanolic solution of scaposin caused an increase in the intensity of band II in the UV spectrum but no bathochromic shift was observed, a result typical for flavones having the 5,7-dihydroxy-6,8-dimethoxy substitution.[2–5] The expected[7] 8–20 mμ bathochromic shift of band II in the presence of fused sodium acetate is not observed with compounds having the 5,7-dihydroxy-6,8-dimethoxy substitution pattern even though they possess an OH group at C-7.

Alkaline degradation of scaposin

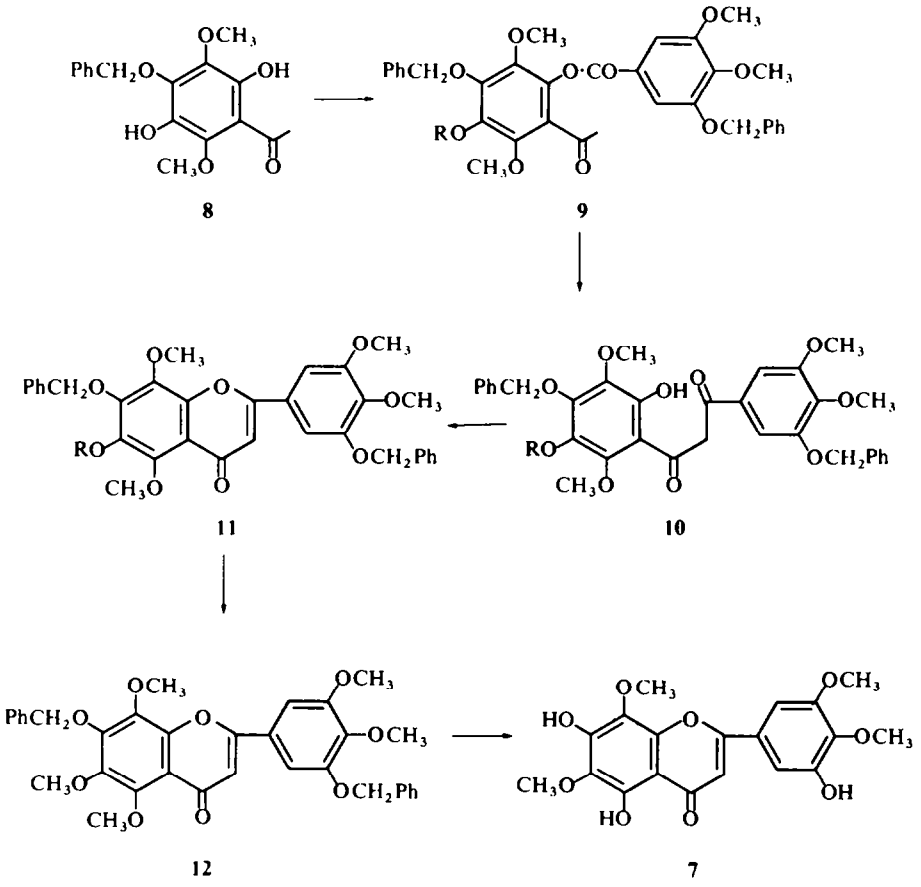
The ring B substitution was shown to be 5'-hydroxy-3',4'-dimethoxy by alkaline degradation of scaposin to 3,4-dimethoxygallic acid, m.p. 193–194°, identical in all respects with a synthetic sample prepared by the method of Späth and Röder.[8] Ring A of scaposin must therefore contain two OH and two OMe groups. The spectral and degradative evidence described above, and biogenetic considerations (co-occurrence of scaposin with hymenoxin and demethoxysudachitin) suggested that scaposin was 5,5',7-trihydroxy-3'4',6,8-tetramethoxyflavone (**7**).

Synthesis of scaposin

The final proof of structure of scaposin was established by synthesis. 4-Benzyloxy-2,5-dihydroxy-3,6-dimethoxyacetophenone[2] (**8**) was converted to **9** on treatment with 5-benzyloxy-3,4-dimethoxybenzoylchloride[8,9] in pyridine. Rearrangement to the dibenzoylmethane derivative **10** (Baker-Venkataraman transformation[10]) followed by ring closure with ethanolic H_2SO_4 afforded the flavone **11**. All attempts to crystallize **11** failed but its PMR spectrum (on material obtained from preparative TLC) indicated that ring closure to the flavone had been achieved [singlet at 6·78 ppm (H-3)]. Saponification of **11** followed by methylation gave 5',7-dibenzyloxy-3',4',5,6,8-pentamethoxyflavone (**12**). Debenzylation and selective demethylation of the C-5 OMe group using refluxing AcOH–HCl yielded 5,5',7-trihydroxy-3',4'6,8-tetramethoxyflavone, which was identical in all respects with scaposin.

Scaposin is of considerable biogenetic interest since it contains both the rare 5,7-dihydroxy-6,8-dimethoxy A-ring system and the unusual if not unique 3',4'-dimethoxy-5'-hydroxy B-ring substitution pattern. The isolation of scaposin, along with hymenoxin[5] (**6**) and demethoxysudachitin (**1**) from *Hymenoxys scaposa*, represents the third report of flavones with the 5,7-dihydroxy-6,8-dimethoxy A-ring substitution pattern from the Family Compositae (the two other reports are nevadensin (**2**)

from *Iva nevadensis*[2] and acerosin (**4**) from *I. acerosa*[3]). Sudachitin (**3**) from *Citrus sudachi*[1] (Family Rutaceae) and lucidin (**5**) from *Lindera lucida* (Family Lauraceae) are the only other flavones which have the same A-ring substitution pattern as found in **1**, **2**, **4** and **6**.



EXPERIMENTAL

M.ps are uncorrected. UV spectra were determined in abs MeOH. Unless otherwise stated, PMR spectra were determined at 60 Mc in $CDCl_3$ solns containing TMS as internal reference. Analyses were determined by Dr. Alfred Bernhardt, Max-Planck Institute für Kohlenforschung, Mülheim, West Germany.

Isolation of scaposin

Dried ground leaves of *Hymenoxys scaposa* DC.[12] (196 g) collected approximately 25 miles northwest of Austin, Texas, April 23, 1966, were extracted with cold petrol (2 l., 48 hr) and then with cold CH_2Cl_2 (three 1-l. portions, 48 hr each). The combined CH_2Cl_2 extracts yielded 13 g of crude gum which was taken up in $CHCl_3$ and chromatographed over silica gel (column 40 cm long, 4·5 cm diam). Elution with $CHCl_3$:MeOH (99·5:0·5) gave in fractions (500 ml each) 6–8, hymenoxin (**6**) as previously described.[5] Further elution with the same solvent yielded crude scaposin (**7**, fractions 10–13), m.p. 195–200°. Rechromatography and recrystallization from $CHCl_3$–C_6H_6 gave fine yellow needles and raised the m.p.

to 210–212°. Scaposin had UV λ_{max} 281 (18,200) and 327 mμ (15,900); λ_{max} (with MeONa) 281, 305 (infl) and 377 mμ; λ_{max} (with $AlCl_3$ and HCl) 292, 303 (infl) and 372 mμ; IR bands (KBr) 1656 (ketone), 1615, 1595 and 1513 (aromatics) cm^{-1}; PMR (DMSO-d_6) 7·25 (singlet, H-2'), 7·20 (singlet, H-6'), 6·90 (singlet, H-3), 7·94, 7·91, 7·83, 7·82 (four singlets, four OMe's) and 12·71 ppm (singlet C_5—OH); PMR of trimethylsilyl ether (CCl_4) 7·07 (singlet, H-2' and H-6') and 6·37 ppm (singlet, H-3); PMR of scaposin 5',7-di-trimethylsilyl ether (CCl_4) 6·48 (singlet, H-3) and 12·40 ppm (singlet, C_5-OH). (Found: C, 58·4; H, 4·4. $C_{19}H_{18}O_9$ requires: C, 58·45; H, 4·6%). Scaposin gave a triacetate which crystallized from MeOH as colorless prisms, m.p. 179–180°.

Further elution of the column afforded in fractions 14 and 15 demethoxysudachitin (**1**, 10 mg), m.p. 260–265°, identical in all respects with an authentic specimen.[11]

Alkaline degradation of scaposin

Scaposin (126 mg) was refluxed with a 50% KOH aq (10 ml) containing a few drops of MeOH for 16·5 hr under N_2. The soln was cooled, acidified with 3N HCl and extracted with ether (2 × 15 ml). The ethereal solution was extracted with 8% $NaHCO_3$ aq (25 ml) and the alkaline extract was acidified and extracted with ether (2 × 15 ml). After removal of the solvent the residue (about 20 mg) was crystallized from hot water yielding white needles, m.p. 193–194°. The compound had PMR and IR spectra, m.p. and m.m.p. identical with a sample of 3,4-dimethoxygallic acid prepared by the method of Späth and Röder.[7]

4-Benzyloxy-3,6-dimethoxy-2,5-di(5-benzyloxy-3,4-dimethoxybenzoyloxy)-acetophenone (**9**)

5-Benzyloxy-3,4-dimethoxybenzoyl chloride (2·7 g) and **8** (0·8 g) were heated on a steam bath in pyridine (3 ml) for 30 min. The mixture was poured into 5% HCl (20 ml) and the oil which separated was extracted with $CHCl_3$ (2 × 10 ml). The residue obtained on evaporation of the $CHCl_3$ soln crystallized from $CHCl_3$–MeOH as colorless prisms, yield 1·2 g, m.p. 146–147°; UV λ_{max} 272 mμ (26,300); PMR 5·21, 5·17, 5·13 (3 singlets, benzyl CH_2 protons), 3·99, 3·97, 3·93, 3·91, 3·83, 3·74 (6 singlets, 6 OMe's), and 2·50 ppm (singlet, CH_3CO—); IR bands (Nujol): 1751 (ketone) and 1587 (aromatics) cm^{-1}. (Found: C, 68·4; H, 5·3. $C_{49}H_{46}O_{14}$ requires: C, 68·5; H, 5·4%).

5'7-Dibenzyloxy-6-(5-benzyloxy-3,4-dimethoxybenzoyloxy)-3',4',5,8-tetramethoxyflavone (**11**)

A mixture of **9** (500 mg), powdered KOH (ca. 60 mg) and pyridine (3 ml) was heated at 60° for 3 hr with stirring. The mixture was poured into 2·5% HCl (15 ml) and the oil which separated was extracted with $CHCl_3$. The residue obtained on evaporation of the $CHCl_3$ was refluxed with 2·5% ethanolic H_2SO_4 (8 ml) for 1 hr. Although TLC showed that the reaction product contained only one major component, all attempts to crystallize the product failed. Preparative TLC gave an oil which had PMR signals at 6·78 (singlet, H-3), 5·28 (singlet, two benzyl CH_2 protons), 5·70 ppm (singlet, benzyl CH_2 protons). The crude material was used in the following step.

5',7-Dibenzyloxy-3',4'-5,6,8-pentamethoxyflavone (**12**)

A mixture of the crude material (**11**) from the previous reaction (about 450 mg) and 1N MeONa (6 ml) was refluxed for 30 min, acidified with AcOH and evaporated to dryness under high vacuum. The residue was refluxed in acetone (10 ml) with Me_2SO_4 (0·8 ml) and K_2CO_3 (2 g) for 1 hr. The inorganic salts were filtered and the solvent removed. The residue crystallized from MeOH as colorless needles, yield 72 mg (23% based on **9**), m.p. 134°; IR bands (KBr): 1642 (ketone), 1591 and 1506 (aromatic) cm^{-1}; PMR 6·58 (singlet, H-3), 5·30, 5·23 (two singlets, two benzyl CH_2 groups), 3·97 and 3·95 ppm (5 OMe protons). (Found: C, 69·7; H, 5·45. $C_{34}H_{32}O_9$ requires: C, 69·9; H, 5·5%).

5,5',7-Trihydroxy-3',4'6,8-tetramethoxyflavone (scaposin, **7**).

Compound **12** (30 mg) was refluxed with AcOH (1·5 ml) and HCl (1·5 ml) for 2 hr. The product was steam distilled and the residue extracted with $CHCl_3$. The flavone crystallized from $CHCl_3$-C_6H_6 (1:1) as fine yellow needles, yield 7 mg (35%), m.p. and m.m.p. with natural scaposin 210–212°. The IR and UV spectra and TLC behavior were identical with those observed for scaposin isolated from *Hymenoxys scaposa*.

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