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CONSTITUENTS OF *IVA* SPECIES—IX

ISOLATION, STRUCTURE PROOF AND SYNTHESIS OF ACEROSIN FROM *IVA ACEROSA* (NUTT.) JACKSON[1,2]

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Abstract—A new flavone, acerosin, has been isolated from *Iva acerosa* (Nutt.) Jackson and identified as 3',5,7-trihydroxy-4',6,8-trimethoxyflavone by synthesis.

IN AN earlier paper,[6] we described the isolation, structure proof and synthesis of the new flavone nevadensin (**1**, 5,7-dihydroxy-4'-6,8-trimethoxyflavone) from *Iva acerosa* (Nutt.) Jackson (copper weed).[7] In addition to nevadensin, another yellow substance, m.p. 239–241°, was obtained in very small amount. Its identification and synthesis is the subject of this paper.

The substance exhibited UV max at 283·5 and 345 mμ and color reactions[9] characteristic of flavonoids. The extremely small amount precluded structural investigation by chemical methods; hence, recourse was taken to physical methods for the determination of the structure. The NMR spectrum (DMSO-d_6) displayed signals at 3·83 (3H) and 3·93 (6H) ppm indicating the presence of three OMe groups. An aromatic AB system at 7·18 d (9) and 7·65 d (9) was attributed to H-5' and H-6' of the flavone nucleus and a singlet at 7·55 to H-2'. The substance also exhibited a singlet at 12·88 ppm characteristic of a bonded OH group at C-5 and another singlet at 6·82 ppm which was attributed to either H-3 or H-8.[10] On the basis of the NMR spectrum, the compound was clearly a trihydroxytrimethoxyflavone containing a OH group at C-5 and the other substituents either in the 3',4',6,7,8- or 3,3',4',6,7-positions.

[1] Work at Florida State University supported in part by a grant from the United States Public Health Service (GM-05814).

[2] Previous paper, W. Herz, H. Chikamatsu, N. Viswanathan and V. Sudarsanam, *J. Org. Chem.* **32**, in press.

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[6] L. Farkas, M. Nógrádi, V. Sudarsanam and W. Herz, *J. Org. Chem.* **31**, 3228 (1966).

[7] This species, previously regarded as the sole representative of the monotypic genus *Oxytenia*, has recently been incorporated into *Iva*.[8]

[8] R. C. Jackson, *U. Kans. Sci. Bull.* **41**, 793 (1960).

[9] K. Venkataraman, *Methods for Determining the Structures of Flavonoid Compounds* (Edited by T. A. Geissman), in *The Chemistry of Flavonoid Compounds* pp. 70–106. Macmillan, New York (1962).

[10] T. Batterham and R. H. Highet, *Austral. J. Chem.* **17**, 428 (1964).

Color reactions and UV spectra offered further insight into the structure of the new flavone. A negative gossypetone reaction indicated that the compound did not contain a free OH group at C-8 and since the color reaction with *o*-dinitrobenzene was negative, it seemed likely that C-6 was substituted by a OMe group.

The change in UV spectrum on addition of reagents used for determining the structure of flavones was quite reminiscent of the changes observed for nevadensin.[6] Just as in the case of nevadensin, which contains a free OH group of C-7, band II of the new substance on addition of fused sodium acetate split into a primary band and an inflection without any shift in the position of the primary band as compared with its position in neutral solution. Although the absence of a bathochromic shift in band II on addition of fused sodium acetate is usually taken to indicate the absence of a free 7-OH group,[11] our experience with nevadensin,[6] a constituent of the same plant which *does* contain a free 7-OH group, suggested that the above behavior suggested the presence of a 7-OH group in acerosin as well.[12] Confirmation of the presence of a OH group at C-5 was obtained by the change in the spectral features on addition of aluminium chloride.

A 50 mμ shift in band I on addition of sodium ethoxide originally suggested the presence of a OH group at C-4′ and, since the spectrum was not affected by the presence of sodium acetate and boric acid, the absence of an *ortho*-dihydroxyl grouping indicated in this manner required that the substituent at C-3′ must be a OMe group.[13] These facts initially led to the suspicion that the second flavone from *Iva acerosa* was 4′,5,7-trihydroxy-3′,6,8-trimethoxyflavone (**2**)[14] or sudachitin, a flavone which had been isolated previously from *Citrus sudachi* Hort. ex Shirai,[17] and whose m.p. coincided with that of our material. The structure of sudachitin has been confirmed by synthesis.[18, 19] However, direct comparison of the flavone from *Iva acerosa* with a sample of sudachitin[20] clearly established non-identity. The mixed m.p. was depressed and while the UV spectra were very similar (see Experimental), there was a significant difference in the shifts and relative intensities of band II in sodium ethoxide–ethanol. The observed shift of 75 mμ in the case of sudachitin was somewhat greater than normal for a free 4′-OH, while the increase in intensity was as expected.[8] On the other hand band II of the new flavone from *Iva acerosa* was

[11] L. Jurd and R. M. Horowitz, *J. Org. Chem.* **22**, 1618 (1957).

[12] A referee has suggested that the anomalous spectral behaviour of nevadensin and acerosin may be a consequence of a (postulated) lower acidity of the 7-OH group in such highly-substituted flavones.

[13] L. Jurd, *Spectral Properties of Flavonoid Compounds*, in Ref. 9, pp. 107–155.

[14] The literature[10] and our own experience[6, 15, 16] indicate that in DMSO-d_6, flavones with a 5,7-dihydroxy framework generally exhibit the H-3 singlet at 6·8–6·9 and the H-8 singlet at 6·5–6·6 ppm. The chemical shift of this singlet in the NMR spectrum of the new compound therefore pointed to **2**, rather than to 4′,5,7-trihydroxy-3,3′,6-trimethoxyflavone. This formulation, in view of the relationship to nevadensin, also seemed more likely on biogenetic grounds.

[15] W. Herz, L. Farkas, H. Wagner, L. Hörhammer and R. Rüger, *Chem. Ber.* **99**, 3539 (1966).

[16] W. Herz and Y. Sumi, *J. Org. Chem.* **29**, 3438 (1964).

[17] T. Horie, M. Masamura and F. S. Okumura, *Bull. Chem. Soc. Japan* **34**, 1547 (1961); *J. Chem. Soc. Japan* **83**, 468 (1962).

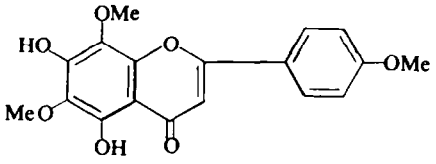
[18] H. H. Lee and C. H. Tan, *J. Chem. Soc.* 6255 (1964). This paper did not come to our attention until the non-identity of sudachitin and acerosin had been established.

[19] L. Farkas and M. Nógrádi, *Acta Chim. Hungarica*, in press.

[20] We wish to thank Dr. T. Horie for supplying an authentic sample.

shifted by a considerably smaller amount (50 mμ) and its intensity was no greater than in neutral solution.

These and the other spectral features could be rationalized by assigning to acerosin, as we have named the new flavone, the formula of 3′,5,7-trihydroxy-4′,6,8-trimethoxyflavone (**3a**), a conclusion which was strongly reinforced by comparison of the NMR spectra of sudachitin and acerosin. The spectra were practically superimposable except for the chemical shifts of the aromatic protons of ring B (sudachitin—7·39s, 7·43d and 6·93d, AB system, $J = 9$; acerosin—7·55s, 7·65d and 7·18d, AB system, $J = 9$).



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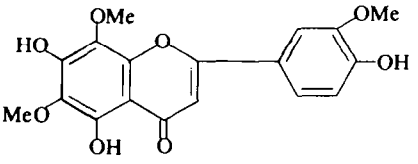


2

To verify the postulated structure of acerosin, the substance was synthesized unambiguously by a series of reactions which resembled the sequence used for the preparation of nevadensin.[6] Acylation of **4a**[6] to **4b** followed by the Baker–Venkataraman transformation[21] furnished 4-benzyloxy-2-hydroxy-5-(3-benzyloxy-4-methoxybenzoyloxy)-4′,3,6-trimethoxydibenzoylmethane (**5**) which was not isolated but cyclized to 3′,7-dibenzyloxy-6-(3-benzyloxy-4-methoxybenzoyloxy)-4′,5,8-trimethoxyflavone (**6a**). Saponification of **6** to **6b** and methylation yielded 3′,7-dibenzyloxy-4′,5,6,8-tetramethoxyflavone (**6c**). Debenzylation and demethylation of **6c** at 20° with hydrochloric acid–acetic acid then afforded 3′,5,7-trihydroxy-4′,6′,8-trimethoxyflavone (**3a**) which was characterized as the triacetate (**3b**) and was identical in all respects with acerosin.

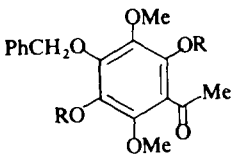
The results of this work again illustrate the caution which must be exercised in placing reliance on spectroscopic rules for structural diagnosis in the flavone series.

EXPERIMENTAL

Isolation of acerosin. The extraction of an *Iva acerosa* (Nutt.) Jackson collection from near Logan, Utah, has been described previously.[6] Chromatography of the crude extract gave,[6] in fractions 22–26 (benzene–$CHCl_3$ 2:1), nevadensin (**2**) and in fractions 32–37 (benzene–$CHCl_3$ 1:1), coronopilin. Fractions 38–42 (benzene–$CHCl_3$ 1:2) gave gums which could not be crystallized. Fraction 43 (benzene–$CHCl_3$ 1:2) gave a semisolid which, when dissolved in a mixture of $CHCl_3$, ether and hexane and allowed to evaporate at room temp, gave 10 mg solid material. This, on repeated recrystallization from $CHCl_3$–ether–hexane, furnished 3–4 mg acerosin, m.p. 239–241°, mixed m.p. with sudachitin greatly depressed. In view of the small amount of material, it was not analysed, but used for color reaction and spectrometric determinations. It exhibited the following color reactions: Mg–HCl—reddish-yellow. NaOH—yellow. $FeCl_3$—green. benzoquinone in EtOH—negative. *o*-dinitrobenzene and $NaHCO_3$aq—negative. UV max 283·5 and 345 mμ (slightly weaker than first band), minima at 264 mμ and 308·5 mμ; with fused AcONa λ_{max}

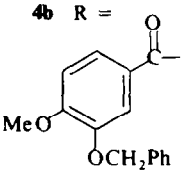
[21] W. Baker, *J. Chem. Soc.* 1381 (1933); K. Venkataraman and H. S. Mahal, *Ibid.* 1767 (1934).

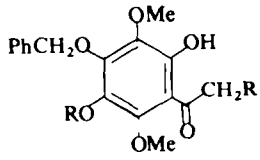
[22] NMR spectra were run on an A-60 instrument in deuteriodimethylsulfoxide using TMS as an internal standard. UV spectra on samples isolated from natural sources were run in abs EtOH on a Cary Model 14 instrument.



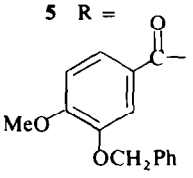
4a R = H

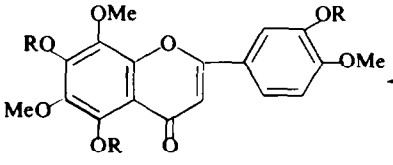
4b R =

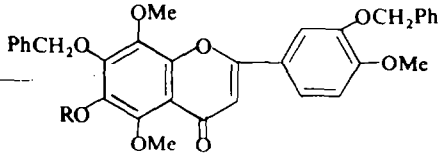




5 R =

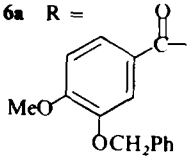




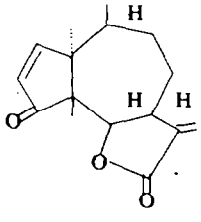


3b R = Ac

6a R =



6b R = H
6c R = Me



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283·5, 315 (infl.) and 381 mμ, λ_{min} 262 and 344 mμ; with $AlCl_3$ λ_{max} 270, 297 and 379 mμ, λ_{min} 259, 281 and 335 mμ; with EtONa 274, 305 (infl.) and 395 mμ (weaker than 274 mμ band), λ_{min} 250 and 323 mμ; with AcONa–boric acid λ_{max} 283·5 and 344 mμ, λ_{min} 265 and 310 mμ.

UV bands of a sample of naturally-occurring sudachitin were as follows: λ_{max} 240 (sh), 283 (20.000), 345 (21.800), λ_{min} 263 (13.100), 307 (13.500), with fused AcONa. λ_{max} 283 (26.900), 315 (sh, 18.900), 381 (18.500), λ_{min} 262 (23.600), 344 (16.600), with EtONa 235 sh (24.000), 270 (sh, 16.000), 285 (19.800), 346 (12.400) and 420 (32.000), λ_{min} 253 (14.500), 309 (5900) and 358 (11.800), with $AlCl_3$ λ_{max} 235 (sh), 255 (sh, 16.000), 262 (17.000), 291 (20.900) and 375 (25.800), λ_{min} 252 (14.900), 270 (16.000) and 334 mμ (10.300).

Further increase in the polarity of the eluting solvent gave only gummy material.

Extraction of a collection of *I. acerosa* made in 1965 near Salt Lake City, Utah, furnished results which differed slightly from those obtained with the Logan collection.[6] We were able to isolate ambrosin (7) in addition to coronopilin and nevadensin, but no acerosin. The crude gum, wt. 66 g, from 1·8 kg of powdered plant was chromatographed over 650 g silicic acid in 1 l. fractions. Fractions 6–10 (benzene) yielded waxy material (5 spots on TLC, mixture of triterpenes, positive Noller test), fractions 11–20 (benzene–$CHCl_3$ 3:1), 21–30 (benzene–$CHCl_3$ 2:1), 31–40 (benzene–$CHCl_3$ 1:1) and 41–44 (benzene–$CHCl_3$ 1:2), yielded gums. Fractions 44–54 (benzene–$ChCl_3$ 1:3) eluted gummy material which solidified on trituration with 1:1 ether–hexane. Recrystallization from acetone–ether–hexane furnished 1·9 g ambrosin, m.p. 144–145°, identical in NMR, mixed m.p. IR with an authentic sample. Fractions 55–60 (benzene–$CHCl_3$ 1:3) gave gum, fractions 61–67 (benzene–$CHCl_3$ 1:3) yielded gummy material which solidified on trituration with 1:1 ether–hexane. The yellow solid was recrystallized from MeOH, double m.p. 186–188° and 193–195°, wt. 0·15 g, identical with nevadensin in IR and NMR spectrum and in mixed m.p. Fractions 68–72 ($CHCl_3$) gave gums; fractions 73–75 gave semisolid material which was recrystallized from acetone–ether and identified as coronopilin, m.p. 175°, wt 0·6 g. Increasing the polarity of the eluates yielded gums from which no acerosin could be isolated, although its presence could not be excluded (TLC).

4-*Benzyloxy*-3,6-*dimethoxy*-2,5-*di*-(3-*benzyloxy*-4-*methoxybenzoyloxy*)*acetophenone* (**4b**). A soln of 0·5 g of **4a**[6] and 1·0 g 3-benzyloxy-4-methoxybenzoyl chloride, freshly prepared from the acid[23] with $SOCl_2$, in 5 ml CH_2Cl_2 was mixed with 0·5 ml dry pyridine, allowed to stand overnight, refluxed for 20 min, washed with dil HCl, dried and evaporated to dryness. The residue was recrystallized from acetone, yield 0·37 g (30%) of **4b**, small colorless rods, m.p. 206–208. (Found: C, 70·25; H, 5·10. Calc. for $C_{47}H_{42}O_{12}$: C, 70·66; H, 5·30%.)

3′,7-*Dibenzyloxy*-6-(3-*benzyloxy*-4-*methoxybenzoyloxy*)-4′,5,8-*trimethoxyflavone* (**6a**). A mixture of 0·3 g of **4b** and 25 mg powdered KOH was heated with 1·5 ml pyridine for 5 min on the steam bath and poured onto 10 ml 5% HCl. The crude yellow **5** which separated was refluxed for 2 hr with 2 ml $CHCl_3$ and 5 ml 2·5% ethanolic H_2SO_4. The crystals which separated on cooling were recrystallized from acetone–CH_2Cl_2, yield of **6a** 0·18 g (60%), colorless needles, m.p. 201–203°. (Found: C, 71·90; H, 5·26. Calc. for $C_{47}H_{40}O_{11}$: C, 72·79; H, 5·16%.)

3′,7-*Dibenzoyloxy*-4′,5,6,8-*tetramethoxyflavone* (**6c**). A mixture of 0·32 g of **6a**, 1 ml $CHCl_3$ and 3 ml 1N MeONa was refluxed for 20 min, acidified to pH 5 with AcOH and evaporated to dryness. The residue was refluxed for 1 hr with 0·5 ml Me_2SO_4 and 10 ml acetone in the presence of K_2CO_3, filtered, evaporated and the residue chromatographed over 15 g silicic acid, solvent benzene–AcOEt (2:1). This afforded 0·14 g pure **6c** (60%). Recrystallization from MeOH gave colorless needles, m.p. 136–138°. (Found: C, 71·72; H, 5·33. Calc for $C_{33}H_{30}O_8$: C, 71·48; H, 5·45%.)

3′,5,7-*Trihydroxy*-4′,6,8-*trimethoxyflavone* (*acerosin*, **3a**). A mixture of 90 mg of **6c**, 4 ml conc. HCl and 4 ml AcOH was refluxed for 2 hr, steam distilled and the crude product which separated on cooling purified by preparative TLC over silicic acid, solvent benzene–pyridine (4:1). Recrystallization from MeOH–AcOEt afforded tiny yellow rods of acerosin, yield 20 mg, m.p. 240–242°, mixed m.p. with material isolated from *Iva acerosa* undepressed. IR spectra and TLC behavior indistinguishable. UV spectrum (EtOH) λ_{max} 250 (infl., 10,300), 218 (12,000), 342 (117,000), λ_{min} 263 (8800), 306 (8500); (0·002N MeONa) λ_{max} 233 (infl., 13,700), 279 (14,200), 308 (infl., 8800), 386 (10,000); λ_{min} 253 (11,000), 329 (6500); (EtOH–$AlCl_3$) λ_{max} 235 (infl., 11,400), 260 (9000), 294 (10,800), 368 (12,700), λ_{min} 250 (7700), 270 (8500), 327 mμ (6000). (Found: C, 59·62; H, 4·80. Calc. for $C_{18}H_{16}O_8$: C, 60·00; H, 4·48%.)

3′,5,7-*Triacetoxy*-4′,6,8-*trimethoxyflavone* (**3b**). Acetylation of 10 mg synthetic acerosin with Ac_2O–AcONa in the usual manner furnished a crude acetate which was purified by preparative TLC over silicic acid, solvent benzene–AcOEt (4:1). This afforded colorless needles, m.p. 131–133°. (Found: C, 59·09; H, 4·50. Calc. for $C_{24}H_{22}O_{11}$: C, 59·01; H, 4·54%.)

Acknowledgement—We wish to thank the Florida State University Research Council for a grant-in-aid to help defray the cost of plant collections.

[23] A. Lovecy, R. Robinson and S. Sugasawa, *J. Chem. Soc.* 817 (1930).