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# FERPRENIN, A PRENYLATED COUMARIN FROM *FERULA COMMUNIS*

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**Key Word Index**—*Ferula communis*; Umbelliferae; prenylated coumarins; ferprenin; ferulenol.

**Abstract**– The structure of ferprenin, a prenylated coumarin from *Ferula communis*, has been established from spectral data, and by synthesis from *E,E*-farnesal and 4-hydroxycoumarin. Ferprenin could also be obtained from ferulenol, the major constituent of the plant investigated, by a $Cr^{6+}$-mediated oxidative cyclization, for which a possible mechanism is proposed.

## INTRODUCTION

Although *Ferula communis* L. was identified as the causative agent of a severe haemorrhagic disease more than 60 years ago [1], the nature of its toxic constitutents is still not entirely clear. Poisoning from *F. communis* causes symptoms in cattle similar to those described for the intoxication by fermented sweet clover, and it was suggested that the plant contains anti-thrombic coumarin derivatives [2]. Ferulenol (**1**), a prenylated 4-hydroxycoumarin isolated from *F. communis* [3, 4], shows haemorrhagic activity, but the toxicity of the plant does not correlate with its contents of (**1**) (Ugazio, G. and Aragno, M., personal communication). The situation is further complicated by a bewildering variability in the secondary metabolism of the plant, due to the presence of several chemical races, some of which contain daucane esters and do not show haemorrhagic activity [4].

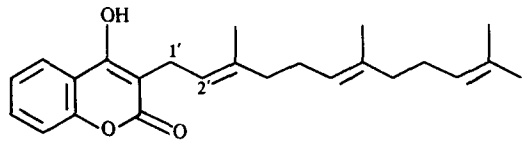
From a sample of *F. communis* collected in Sardinia, where the disease was first described and is still widespread in cattle, we have isolated a new prenylated coumarin (**2**), ferprenin.

## RESULTS AND DISCUSSION

Ferprenin was isolated as an optically active colourless oil of molecular formula $C_{24}H_{28}O_3$ (mass spectrometry). Its UV spectrum was distinctly different from that of ferulenol (**1**), with the maximum at 310 nm shifted at 355 nm as the result of the presence of a more extended chromophore. Compared to that of **1**, the $^1$H NMR spectrum of **2** showed the presence of an isolated olefinic AB system ($\delta$6.59, *d*; 5.49, *d*; $J = 10.1$ Hz) in place of the $A_2X$ system of the protons at C-1′ and C-2′, and the substitution of a methyl bound to a quaternary centre ($\delta$1.53, sharp *s*) for an allylic methyl. These data could be rationalized by the pyrano[3,2-*c*]coumarin structure (**2**) for ferprenin. This was also substantiated by $^{13}$C NMR (see Experimental) and mass spectroscopy. In the mass spectrum, the base peak at *m/z* 213 was assigned to the pyrilium ion **A**, resulting from allylic cleavage between C-3′ and C-4′. The stereochemistry at the C-6′–C-7′ dobule bond was established as *E* from the upfield position of the methyl bound to C-7′ ($\delta$15.90, *q*) [4]. No attempt was made to establish the stereochemistry of the quaternary centre C-3′.

The structure of **2** was confirmed by obtaining racemic ferprenin in 64% yield from the reaction of *E, E*-farnesal and 4-hydroxycoumarin, according to a general entry for 2*H*-pyranes anellated to aromatic rings already applied to 4-hydroxyquinolone, which is isoster with 4-hydroxycoumarin [5]. The reaction is a tandem Knoevenagel electrocyclic rearrangement, proceeding via a quinone methide-type *cis*-dienone intermediate (Scheme 1). Ferprenin could also be obtained from the treatment of ferulenol with a variety of $Cr^{6+}$-based reagents. The formation of pyrano[2,3-*c*]coumarins seems to be general for 4-hydroxycoumarins bearing an allylic residue at C-3, as we accidentally discovered during the oxidation of isomeric hydroxyferulenols. This reaction is similar to the DDQ-mediated oxidation of some *ortho*-allylphenols to 2*H*-chromenes, which is believed to comply with the proposed hypothesis for the biogenesis of 2*H*-chromenes, and to occur via a *cis*-dienone intermediate [6]. The oxidation of ferulenol to ferprenin presumably takes place through the formation of a $Cr^{6+}$ ester, followed by the loss of a $Cr^{4+}$ species *via* the vinylogous version of the process postulated for the PCC oxidation of alcohols [7] (Scheme 1). This would give the same dienone formed during the condensation of 4-hydroxycoumarin and farnesal. Among the $Cr^{6+}$ reagents tested (Jones, PCC, PDC), PDC gave the best yield (45%).

Ferprenin is unstable in solution (see Experimental), and so, although its isolated yield was low (0.043% on dried plant material, only 10% of that of ferulenol), the actual concentration in the plant might be higher. We are at present investigating whether ferprenin, which bears a certain similarity with ciclocumarol, is one of the toxic agents in *F. communis*.



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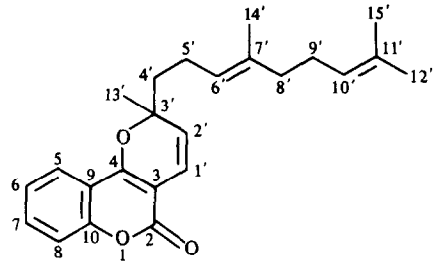

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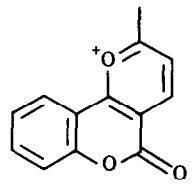

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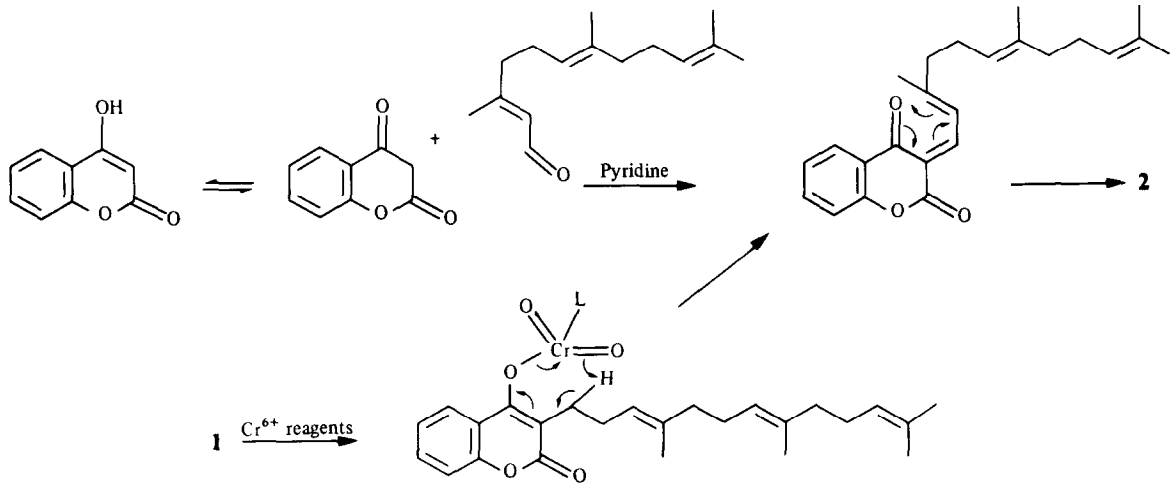

Scheme 1. Synthesis of ferprenin (L = -OH/ -Cl/ $-OCrO_3^-$)-

## EXPERIMENTAL

Silica gel 60 (70–230 mesh, Merck) was used for CC; a Water microporasyl column (8 × 30 cm) was used for prep. HPLC, using a Water 501 pump coupled with a Water differential refractometer R 401.

*Plant material. F. communis* was collected in November 1986 at Olliena (NU), and was identified by V. P.

*Isolation of ferprenin.* 1.7 kg of dried roots were coarsely powdered, and extracted with $CH_2Cl_2$ (3 × 5 l) at room temp. Removal of the solvent left 80 g of extract (4.7%), a part of which (10.8 g) was defatted by dissolving it in MeOH and cooling in the fridge for 2 weeks. After filtration of the precipitate (fluted filter paper), the clear filtrate was evapd. The residue was partioned between 5% $Na_2CO_3$ and $CH_2Cl_2$, to give 1.08 g of acidic fraction and 8.3 g of neutral fraction. The latter was separated by CC on silica gel (100 g), using mixtures of hexane and EtOAc as eluents. From the fractions eluted with hexane–EtOAc (4:1), 407 mg of a brownish oil were obtained, which was further purified by prep. HPLC [hexane–EtOAc (4:1) as eluent] to give 98 mg of ferprenin (yield: 0.043%).

*Ferprenin*, (2H, 5H, -2-*methyl*-2-(4,8-*dimethylnona*-3,7-*dienyl*)-*pyrano* [3,2-c] [1] *benzopyran*-5-*one*) (**2**). Colourless oil, $[\alpha]_D^{25}$ +10 ($CHCl_3$, *c* 0.9); IR $\nu_{max}^{liquid\ film}$ $cm^{-1}$: no -OH band, 1720, 1645, 1610, 1365, 1040, 910, 760, 730; UV $\lambda_{EtOH}^{max}$ (log ε) nm: 375 (sh), 355 (4.1), 343 (4.2), 246 (4.4); EIMS 70 eV, *m/z* (rel. int.): 364 $[M]^+$ (35); 295 $[M-C_5H_9]^+$ (16); 214 (30); 213 $[A]^+$ (100); 175 (15); 121 (23); 107 (16); 69 $[C_5H_9]^+$ (65); 41 (35). $^1H$ NMR (270 MHz, $CDCl_3$, TMS as reference): $\delta$ 7.80 (*dd*, $J_{5,6} = 7.8$; $J_{5,7} = 1.8$ Hz, H-5); 7.53 (*dq*, $J_{5,6} = 7.8$; $J_{6,7} = 8.5$; $J_{6,8} = 1.8$ Hz, H-6); *ca* 7.30 (*m*, H-

6+H-8); 6.59 (*d*, $J_{1',2'}$ = 10.1 Hz, H-1′ or H-2′); 5.49 (*d*, $J_{1',2'}$ = 10.1 Hz, H-2′ or H-1′); 5.08 (*br m*, H-6′+H-10′); 1.67 (*br s*, H-12′); 1.58, 1.56 (*br s*, H-14′ and H-15′); 1.53 (*s*, H-13′). $^{13}$C NMR (67.80 MHz, $CDCl_3$, TMS as reference): C-2: δ161.80 *s*; C-3: 99.97 *s*; C-4: 159.40 *s*; C-5: 115.47 *s*; C-6, C-7: 122.56 *d* and 123.13 *d*; C-8: 131.90 *d*; C-9: 116.68 *d*; C-10: 153.19 *s*; C-1′, C-2′: 125.03 *d* and 117.24 *d*; C-3′: 83.10 *s*: C-4′: 41.76 *t*; C-5′: 22.35*t*; C-6′ and C-10′: 123.83 *d* and 124.15 *d*; C-7′: 135.89 *s*; C-8′: 39.54 *t*; C-9′: 26.57 *t*; C-11′: 131.25 *s*; C-12′: 25.55*q*; C-13′: 27.40 *q*; C-14′: 15.90 *q*; C-15′: 17.57 *q*. Ferprenin decomposes if kept in solution at room temperature in a variety of solvents ($CHCl_3$, $CH_2Cl_2$, $Me_2CO$). A certain decomposition takes also place if solutions are kept in the fridge, as judged by TLC. The pure product could be stored in the fridge for months without decomposition.

*Synthesis of ferprenin.* (i) From E,E-farnesal: a soln. of *E,E*-farnesal [prepared by PDC oxidation of *E,E*-farnesol (Aldrich)] (1.337 g, 6.07 mMol) and dry $MgSO_4$ (1.910 g) in 3 ml dry pyridine (distilled from $CaH_2$) was brought to boiling. Then, under $N_2$, a soln of 4-hydroxycoumarin (0.984 g, 6.07 mMol) in 6 ml dry pyridine was added dropwise over 1 hr, maintaining refluxing. After cooling, the reddish soln was dild with water and extracted with $CH_2Cl_2$. The organic phase was washed with satd $CuSO_4$ and dried ($MgSO_4$). Evaporation of the solvent gave 1.936 g of crude ferprenin, which was further purified by CC on a short column of silica gel (7 g) eluted with hexane–EtOAc 9:1. 1.41 g of racemic ferprenin were obtained (yield: 64%).

(ii) From ferulenol. To a suspension of PDC (310 mg, 0.82 mMol, 1.5 mol. equiv.) in 0.4 ml dry $CH_2Cl_2$ (distilled from $P_4O_{10}$), a soln of ferulenol (200 mg, 0.55 mMol) in 0.7 ml $CH_2Cl_2$ was added dropwise. The mixture was stirred at room temp. and the course of the reaction was followed by TLC (hexane–EtOAc 6:4; $R_f$ ferulenol: 0.45; $R_f$ ferprenin: 0.64). After 3.5 hr, all ferulenol had reacted, and the reaction was worked up by the addition of 400 mg Celite® and 15 ml ether. The suspension was filtered through a fluted filter paper, and the filtrate was evaporated and purified by CC (4 g silica gel, hexane–EtOAc 9:1 as eluent), to give 89 mg of racemic ferprenin (yield: 45%).

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# A DIHYDROFLAVONOL-*O*-GLYCOSIDE OF *CITRUS SINENSIS*

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**Key Word Index**—*Citrus sinensis*: Rutaceae; dihydroflavonol glycoside; dihydrokaemferol-4′-methyl ether-7-*O*-rhamnoside.

**Abstract**—Dihydrokaenferol-4′-methyl ether-7-*O*-rhamnoside has been isolated from the pulp of mature fruit of *Citrus sinensis*.

## INTRODUCTION

The genus *Citrus* is a rich source of various types of flavanoid compounds. In this paper we report on the isolation and characterization of dihydrokaemferol-4′-methyl ether-7-*O*-rhamnoside from the pulp of mature fruit of *C. sinensis* L.

## RESULTS AND DISCUSSION

The compound gave a magenta colour in the Shinoda test. Its IR spectrum (Nujol) showed a very broad band at 3200–3500 $cm^{-1}$ which indicated the presence of a sugar moiety. There were also strong bands at 1655 $cm^{-1}$ for a carbonyl group and a band at 1615 $cm^{-1}$ (aromatic ring).