Trigocoumarin – a New Coumarin from *Trigonella foenumgraecum*

Virinder S. Parmar*, Hirday N. Jha, Sudershan K. Sanduja, and Radhika Sanduja
Department of Chemistry, University of Delhi, Delhi-110007, India

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A new coumarin, named trigocoumarin has been isolated from *Trigonella foenumgraecum* along with some known compounds. Based upon its spectral (IR, UV, NMR and MS) and analytical data, trigocoumarin has been assigned the structure: 3-(ethoxycarbonyl)-methyl-4-methyl-5,8-dimethoxycoumarin.

Introduction

Trigonella foenumgraecum Linn. (Leguminoseae, sub family Lotoideae) is an annual herbaceous plant widely distributed in Asia, Africa and Europe. In India and other countries its seeds are commonly used as a spice and medicine, in particular, for the treatment of colic, dysenetry, diarrhoea, dyspepsia and chronic cough [1, 2]. Various parts of this plant from different countries have been studied for their chemical components and the types of compounds occurring in this species are stereoidal sapogenins [3, 4], carotenoids [5], coumarins [6] and flavonoids [7–9]. The present communication deals with the isolation and structure elucidation of a new coumarin besides the earlier known compounds.

Results and Discussion

The whole plant was extracted, fractionated and chromatographed as described in the Experimental section. Various fractions collected from column chromatography were purified by preparative TLC followed by crystallisation to afford twelve pure compounds, eleven of which were in sufficient quantities to allow complete identification. Work is in progress for the isolation and structure elucidation of the other compound. With the help of their physical and spectroscopic data and also by comparison with the corresponding authentic samples, ten compounds were found to be already known from the source; they are β-sitosterol, β-sitosterol-D-O-glucoside (isolated for the first time from this source), diosgenin, *p*-coumaric acid, 4-methyl-7-acetoxycoumarin, 7,4′-dimethoxyflavanone, luteolin, quercetin, vitexin and isovitexin. The characterisation of the remaining compound (trigocoumarin) is described.

Trigocoumarin ($C_{16}H_{18}O_6$ from analytical data and M^+ peak at *m/e* 306 in its MS) was obtained as colourless needles, mp 87–88 °C. It gave a blue fluorescence in UV light and furnished yellowish-orange colour with NaOH; it did not respond to any metal/acid reduction tests. The IR spectrum showed strong absorptions at 1605, 1700 and 1730 cm^{-1}; these colour reactions and spectral data are attributable to a coumarin system. Its NMR spectrum ($CDCl_3$) showed signals for two methoxyl groups, an ethoxyl group, a C-methyl group, a methylene group (flanked by a carbonyl group and an ene group) and two ortho coupled aromatic protons at δ 6.89 and 7.35 ($J = 9$ Hz each). The absence of characteristic signals for the protons at C-3 and C-4 positions of a coumarin system in its NMR spectrum indicated that both these positions are substituted in trigocoumarin. By studying the NMR spectra of a number of synthesized 4-methyl coumarins, it has been found that the methyl group in C-4 methyl coumarins invariably appears around δ 2.35–2.4 [10]. As the C-methyl group in trigocoumarin appeared at δ 2.38 in its NMR spectrum, it was indicated that it carries a methyl group at C-4; moreover a 4-methyl coumarin has earlier been isolated from this plant [6]. Very close chemical shift values (δ 3.95 and 3.96) of the two methoxyl groups indicated that both are located in the aromatic ring; coupled with the nature of the remaining two aromatic proton signals (as ortho-coupled doublets), the two methoxyl groups could be at 5, 6; 5, 8 or 7, 8 positions. Grigg *et. al.* [10] have studied the benzene-induced solvent shifts of C-4 methyl signal in a number of synthetic coumarins and have concluded that in the absence of a 5-methoxyl group, this shift is 0.63–0.8 ppm while in the presence of such a group it is reduced to

* Reprint requests to V. S. Parmar.
Present address: St. Stephen's College, Delhi-110007, India.

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0.38–0.4 ppm only. As the benzene-induced upfield shift of the C-4 methyl group in trigocoumarin was only 0.35 ppm, one methoxyl group was inferred to be located at C-5 position. Further, it has been noted by us [11, 12] that the C-7 and C-8 protons in 4,5,6-trisubstituted coumarins appear as a singlet at *ca.* δ 7.1, thus indicating that the two methoxyl groups are present at C-5 and C-8 positions in trigocoumarin. A strong absorption at 1730 cm^{-1} in its IR spectrum, a singlet at δ 3.6 for a methylene group and signals for an ethoxyl group in its NMR spectrum are attributable to the presence of $-CH_2-\overset{\overset{O}{\|}}{C}-OC_2H_5$ group at the C-3 position in the compound. From the above detailed analysis of its IR and NMR spectra, trigocoumarin could be assigned the structure, 3-(ethoxycarbonyl)methyl-4-methyl-5,8-dimethoxycoumarin. This structure is fully compatible with the fragmentation pattern observed in its mass spectrum, and the benzene-induced solvent shifts of the methoxyl signals in its NMR spectrum [10]. To our knowledge, this is a new compound and thus is being reported for the first time from any natural source and we propose the name trigocoumarin for this. It may also be mentioned that this is the first report of a 5,8-dioxygenated coumarin in a natural source.

Experimental

The IR spectra were recorded on a Perkin-Elmer infracord-137 spectrophotometer in KBr pellets while UV spectra were recorded on a Beckmann DU-2 Spectrophotometer in methanol. The NMR spectra were obtained on a Varian A-60 instrument with TMS as internal standard. The mass spectral analysis were carried out on a Varian MAT CH7 instrument at 100 μA and 70 eV with direct inlet. Melting points were determined in sulphuric acid bath and are uncorrected. For column chromatography silica gel-G (90–200 mesh, E. Merck) was used while TLC was carried out on 0.5 mm silica gel-G (type 60, E. Merck) layers. The whole plant of *Trigonella foenumgraecum* (Leguminoseae) was purchased from the local market of Delhi and identified in the Department of Botany, University of Delhi.

Isolation

The air-dried whole plant (3 kg) was extracted with ethanol (4 × 5 l) in hot. The combined extract was concentrated and the solvent-free residue was extracted repeatedly first with petrol (60–80 °C) and then with ether, when a brownish semi solid type residue (*ca.* 15 g) was left. This residue was chromatographed and the column eluted with mixtures of benzene:ethyl acetate and ethyl acetate:methanol in varying proportions, when diosgenin, β-sitosterol-D-O-glucoside, vitexin and isovitexin were obtained from different fractions. The petroleum ether soluble portion of the plant extract yielded only one crystalline compound (identified as β-sitosterol) in addition to a large amount of greenish gummy waxy mass. A brownish-yellow solid (12 g) was obtained on removing the solvent from the ether soluble fraction of the plant extract. This solid was subjected to column chromatography and the column eluted with mixtures of petrol-benzene and benzene-ethyl acetate. The earlier column fractions contained β-sitosterol and 7,4′-dimethoxyflavanone. The fractions obtained by elution with petrol-benzene (4:1, 1:1, 1:3 and 1:9) afforded a mixture of 7,4′-dimethoxyflavanone, trigocoumarin and 4-methyl-7-acetoxycoumarin. This mixture was separated into pure components by preparative TLC using the solvent system, benzene:methanol (99:1). Fractions eluted by benzene-ethyl acetate (9:1) yielded *p*-coumaric acid in pure form while fractions obtained from benzene-ethyl acetate (3:1, 1:4 and 1:9) contained luteolin and quercetin. These were separated by preparative TLC using benzene:ethyl acetate (4:1) as solvent system.

The known compounds were identified by degradative studies and from their physical and spectral data. The identities were confirmed by preparation of their derivatives and comparisons with the corresponding authentic samples. In the intermediate column fractions of the ether soluble portion, another compound was obtained in minute amounts; further work on this compound is in progress.

Trigocoumarin crystallised from methanol as colourless needles (25 mg); m.p. 87–88 °C: UV λ_{max} (nm): 255, 280 and 310.

IR ν_{max} (cm^{-1}): 1730, 1700, 1605, 1505, 1455, 1350, 1335, 1292, 1215, 1185, 1138, 1112, 1020, 974, 900, 865, 803, 781 and 769.

MS *m/e* (%): 306(100), 291(50), 278(15), 260(22), 259(88), 258(90), 248(28), 234(99), 233(96), 232(72), 205(28), 190(29), 160(39), 150(25), 91(50).

^{1}H NMR data (in $CDCl_3$): δ (ppm) = 7.35 (1H, d, J = 9 Hz, C-7 H), 6.89 (1H, d, J = 9 Hz, C-6 H), 4.19 (2H, q, J = 7 Hz, $-CH_2-COO-C\underline{H}_2CH_3$), 3.96 (3H, s, $-OCH_3$), 3.95 (3H, s, $-OCH_3$), 3.60 (2H, s, $-C\underline{H}_2-COOCH_2CH_3$), 2.38 (3H, s, C-4 CH_3), 1.26 (3H, t, J = 7 Hz, $-CH_2-COOCH_2C\underline{H}_3$).

^{1}H NMR data (in $CDCl_3 + C_6H_6$): δ (ppm) = 3.56 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 2.03 (3H, s, C-4 CH_3).

Analysis for $C_{16}H_{18}O_6$

Found	C 62.36	H 6.12,
Calcd	C 62.74	H 5.88.

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