Phytochemistry, Vol 28, No 4, pp 1229 1231, 1989
Printed in Great Britain

0031 9422/89 $3 00+0 00

COUMARINS FROM THE ROOTS OF *FERONIA LIMONIA*

AMULAYA AGRAWAL, I. R. SIDDIQUI and J. SINGH

Department of Chemistry, University of Allahabad, Allahabad 211 002, India

(*Received* 30 *September* 1988)

Key Word Index—*Feronia limonia*, Rutaceae, roots, coumarins

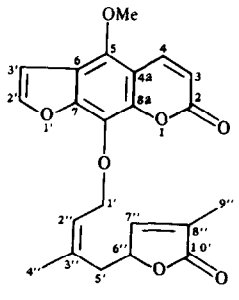
Abstract—A new monoterpenoidfuranocoumarin lactone (fernolin) along with aurapten, marmesin, bergapten and xanthotoxin has been isolated from the roots of *Feronia limonia*. The structure of each of these compounds has been established on the basis of chemical reactions and spectral studies.

INTRODUCTION

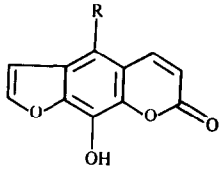
Feronia limonia Swingle (syn *F elephantum* Correa), a common Indian tree, belongs to the tribe Citreae and subtribe Balsamocitrinae [1]. *Feronia*, a single species genus, is found throughout the plains of India, particularly in dry situations. The plant is well known for its medicinal properties The roots are prescribed in the treatment of snake-bite. The different parts of *F. limonia* have been investigated by several workers and found to contain coumarins [2], alkaloids [3], steroids [4], flavonoid glycosides [5] and essential oils [3]. A flavanone glycoside [6] has also been earlier reported from this laboratory. The present investigation on the roots of *F. limonia* revealed the presence of a new coumarin (**1**) along with some known coumarins, i.e. aurapten, marmesin, bergapten and xanthotoxin. Furanocoumarins show photosensitizing effects [7] and xanthotoxin and bergapten are used for the treatment of leucoderma, characteristic of vitiligo [8]

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{22}H_{20}O_7$ ($M^+ = m/z$ 396). Its UV spectrum, λ_{max} 220, 252, 276 and 312 nm, was characteristic of a linear furanocoumarin [9] and no shift was observed on addition of alkali IR absorptions at 1755 and 1710 cm^{-1} indicated the presence of α,β-unsaturated γ-lactone and α,β-unsaturated δ-lactone groups, respectively.



1



1a R = OMe
1b R = OH

On treatment with concentrated sulphuric acid for 10 min at 40°, it afforded compound **1a** as a major product whereas for 30 min, compound **1b** was the only product and on treatment with boron tribromide at 0°, compound **1** yielded only compound **1b** Compound **1a** was identified as 5-methoxy-8-hydroxy psoralen on the basis of mp, mmp [10], UV and ^{1}H NMR and compound **1b** was found to be 5,8-dihydroxypsoralen on direct comparison with an authentic sample [10] On the basis of above evidences, it is established that the compound **1a** was present as the nucleus in the compound **1** or compound **1** was an ether derivative of **1a**.

The 200 MHz spectrum of **1** integrated for 20 protons. A pair of doublets at δ6.35 and 7 76 (J = 9.5 Hz) was attributed to the pyran ring protons at C-3 and C-4, respectively. Another pair of doublets appeared at δ7.71 and 6.80 (J = 2.3 Hz) corresponding to the 2'- and 3'-furan ring protons. A doublet of two protons δ5.09 (J = 6.6 Hz) was assigned to the $-OCH_2-$group at C-1", the methine protons appeared as a multiplet at δ5.72 and the 5"methylene group was observed as a multiplet at δ2.36. The multiplet centred at δ4.92 (J = 6.5 and 1.95) was assigned to the proton at C-6" of a five-membered lactone. The triplet at δ1 88 (J = 1.71 and 1 95 Hz) for three protons could be assigned to the methyl function at the α-position of the α,β-unsaturated γ-lactone group. The three methyl protons at C-4" appeared as a broad singlet at δ1.79 Thus, the structure of fernolin has been represented as **1**. The size and nature of the side chain was also assessed by a study of its mass spectrum [11].

The proposed structure of **1** was confirmed by ^{13}C NMR [12] and decoupling experiments. The relationship of H-9" with H-6" and H-7" was established by decoupling experiment. Irradiation at δ1.8866 caused the multiplet at δ6.93 (*dq*, H-7") to change to a doublet accompanied by a simultaneous change in the shape of the multiplet at δ4.92 (*tm*, H-6"). Irradiation at δ2.3648 simplified the multiplets at δ4.92 (H-6") and 6.93 (H-7") establishing the relationship between H-5", H-6" and H-7". This was further supported by the appearance of sharp singlet at δ1.88 (H-9"), a *dd* at δ2 36 (H-5") and simplification of the multiplet at δ6.93 (H-7") on irradiation at δ4.9254. Irradiation at δ5.0416 simplified the multiplet at δ5.72 (H-2") showing the relationship between H-1" and H-2" supported by the change of doublet at δ5.09 (H-1")

on irradiation at δ5 7267 Irradiation at δ6.9342 changed the *dd* appearing like a triplet at δ1.88 (H-9″) to a doublet accompanied by a simultaneous change in the shape of the multiplet at δ4.92 (H-6″) This compound is new and has not been reported earlier from any natural source

A second compound was analysed for $C_{19}H_{22}O_3$ Its UV absorption maxima was similar to that of mono-alkoxy coumarin [13]. The appearance of IR absorptions at 1725 and 2900–3000 cm^{-1} suggested the presence of α,β-unsaturated δ-lactone (α-pyrone) and *gem*-dimethyl groups, respectively.

The 60 MHz ^{1}H NMR spectrum of this coumarin displayed signals for 7-alkoxycoumarin and a geranyloxy or neryloxy side chain as substituent at C-7 [2]. The geometry about the double bond (2′-3′-position) has been suggested to be *trans* (geranyloxy form) rather than *cis* (neryloxy form) on the basis of fine splitting of the 2′-vinyl hydrogen At 60 MHz, the vinyl proton at C-2′ appeared as a triplet at δ5 48 (1H, J = 6.8 Hz) But at 300 MHz, this absorption appeared as a triplet (J = 6 8 Hz) with each peak further splitting into quartets (J = 1.2 Hz). This splitting and coupling constant along with stereochemical considerations and reported *trans* arrangement of the methyl group to hydrogen in geraniol [14], suggested that the vinyl proton was *trans* to the methyl group. The nature and position of the side chain was also confirmed with the help of ^{13}C NMR [15] and mass spectral evidences From the foregoing evidences, the structure of the compound could be identified as 7-geranyloxycoumarin (aurapten) This known compound was isolated in order to establish its geometry, a controversial point about its structure. The structures of marmesin [16], bergapten [5], and xanthotoxin [2] have also been established on the basis of chemical and spectral evidences

EXPERIMENTAL

The air-dried and crushed roots (5 kg) were extracted with boiling EtOH and the concd ethanolic extract was poured into ice-cold H_2O to separate H_2O-soluble and insoluble fractions The concentrated H_2O-soluble fraction was fractionated with different organic solvents of increasing polarity in a liquid–liquid extractor From the C_6H_6 extract, marmesin and from the ethyl acetate extract compound **1** have been isolated

The H_2O-insoluble fraction was successively extracted with hexane, C_6H_6, Et_2O and EtOAc in a Soxhlet extractor Aurapten, bergapten and xanthotoxin were isolated by prep TLC (benzene–EtOAc, 9 1) from the C_6H_6 extract

Compound **1** (*fernolin*) Mp 262°, UV λ_{max}^{MeOH} nm 220, 252, 276, 312, IR ν_{max}^{KBr} cm^{-1} 2920, 2800, 1755, 1710, 1615, 1590, 1440, 1400, 1325, 1290, 1210, 1180, 1150, 1025, ^{1}H NMR ($CDCl_3$, 100 MHz) δ1 79 (*br s*, 3H, C-4″), 1 88 (*t*, 3H, J = 1 71 and 1 95 Hz, C-9″), 2 36 (*m*, 2H, J = 6 5 Hz, C-5″), 3 90 (*s*, 3H, –OMe), 4 92 (*tm*, 1H, J = 6 5 and 1 95 Hz, C-6″), 5 0 (*d*, 2H, J = 6 6 Hz, C-1″), 5 72 (*tm*, 1H, J = 6 6 and 0 98 Hz, C-2″), 6 35 (*d*, 1H, J = 9 5 Hz, C-3), 6 80 (*d*, 1H, J = 2.3 Hz, C-3′), 6 93 (*dq*, 1H, J = 1 71 and 0 16 Hz, C-7″), 7 71 (*d*, 1H, J = 2 3 Hz, C-2′), 7 76 (*d*, 1H, J = 9 5 Hz, C-4), ^{13}C NMR δ160 37 (C-2), 114 76 (C-3), 144 32 (C-4 and C-7″), 116 58 (C-4a), 131 0 (C-5), 125 95 (C-6), 148 26 (C-7), 131 48 (C-8), 148 65 (C-8a), 146 70 (C-2′), 106 85 (C-3′), 69 69 (C-1″), 123 94 (C-2″), 137 02 (C-3″), 10 58 (C-4″), 43 37 (C-5″), 79 54 (C-6″), 130 8 (C-8″), 17 24 (C-9″), 173 82 (C-10″), 63 00 (–OMe), MS, *m/z* 396 $[M]^+$, 309, 232, 204, 165, 97, 69, 41

Dealkylation of compound **1** *using conc* H_2SO_4 Compound **1** (0 1 g) in HOAc (1 ml) was heated at 40° with conc H_2SO_4 (2 drops) for 10 and 30 min to yield compounds **1a** and **1b**, respectively

Dealkylation of compound **1** *using* BBr_3 A well-stirred soln of compound **1** (0 05 g) in CH_2Cl_2 (20 ml) was treated with BBr_3 (0 05 g) in CH_2Cl_2 at 0° and stirred at room temp for 24 hr On crystallization the product 5,8-dihydroxypsoralen, mp 209°, was obtained

Compound **1a** (*5-methoxy-8-hydroxypsoralen*) Mp 214°, UV λ_{max}^{MeOH} nm 220, 242 (sh), 250, 314, ^{1}H NMR ($CDCl_3$, 90 MHz) δ6 20 (*s*, 1H, –OH), 4 25 (*s*, 3H, –OMe), 6 30 (*d*, 1H, J = 9 Hz, C-3), 6 81 (*d*, 1H, J = 2 3 Hz, C-3′), 7 68 (*d*, 1H, J = 2 3 Hz, C-2′), 7 76 (*d*, 1H, J = 9 Hz, C-4)

Compound **1b**, (*5,8-dihydroxypsoralen*) Mp 210°

Aurapten Mp 66°, UV λ_{max}^{MeOH} nm 245, 254, 324, IR ν_{max}^{KBr} cm^{-1} 3000–3100, 2900–3000, 1725, 1610, 1400, 1350, 1235, 1200, 1125, ^{1}H NMR ($CDCl_3$, 60 MHz) δ1 60 and 1 65 (*s*, 3H each, *gem*-dimethyl group at C-7′), 1 75 (*s*, 3H, 3′-Me), 2 0–2 20 (*m*, 4H, 4′-$>CH_2$ and 5′-$>CH_2$), 4 60 (*d*, 2H, J = 6 5 Hz, 1′-$>CH_2$), 5 -5 2 (*br m*, 1H, 6′-$>CH$ coupling with vicinal 5′-$>CH_2$ and allylic 7′-Me's), 5 48 (*t*, 1H, J = 6 5 Hz, 2′-$>CH$ coupling with 1′-$>CH_2$ and also with allylic 4′-$>CH_2$ and 3′-Me) 6 25 (*d*, 1H, J = 9 5 Hz, C-3), 6 85 (*m*, 2H, C-6 and C-8), 7 37 (*d*, 1H J = 8 5 Hz, C-5), 7 65 (*d*, 1H, J = 9 5 Hz, C-4), ^{13}C NMR δ161 2 (C-2), 112 4 (C-3), 143 4 (C-4), 112 3 (C-4a), 128 6 (C-5), 113 2 (C-6), 162 2 (C-7), 101 6 (C-8), 155 9 (C-8a), 65 5 (C-1′), 118 4 (C-2′), 142 3 (C-3′), 39 5 (C-4′), 26 2 (C-5′), 123 6 (C-6′), 131 9 (C-7′), 25 6 (C-8′), 17 7 (3′-Me), 16 7 (7′-Me), MS, *m/z* 298 $[M]^+$, 229, 163, 161, 162, 137, 136, 134, 106, 69

Marmesin Mp 189°, UV λ_{max}^{MeOH} nm 225, 250, 260, 300 (sh), 337, IR ν_{max}^{KBr} cm^{-1} 3440, 3000, 1700, 1628, 1565, 1485, 1445, 1362, 1128, 960, 840, ^{1}H NMR ($CDCl_3$, 90 MHz) δ1 22 (*s*, 3H, –Me), 1 36 (*s*, 3H, –Me), 3 20 (*d*, 2H, J = 9 Hz, Ar–CH_2–$CH<$), 4 74 (*t*, 1H, J = 9 and 9 Hz, Ar–CH_2–$CH<$), 6 20 (*d*, 1H, J = 9 Hz, C-3), 6 74 (*s*, 1H, C-8), 7 22 (*s*, 1H, C-5), 7 60 (*d*, 1H, J = 9 Hz, C-4), 2 20 (*br s*, 1H, –OH), MS, *m/z* 246 $[M]^+$, 228, 213, 188, 187, 166

Bergapten Mp 186°, UV λ_{max}^{MeOH} nm 220, 250, 261, 266, 309, IR ν_{max}^{KBr} cm^{-1} 3100, 1700, 1625, 1570, 1540, 1450, 1385, 1280, 1123, 880, 810, 735, ^{1}H NMR ($CDCl_3$, 90 MHz) δ4 26 (*s*, 3H, –OMe), 6 40 (*d*, 1H, J = 9 Hz, C-3), 6 83 (*d*, 1H, J = 2 Hz, C-3′), 7 15 (*s*, 1H, C-8), 7 68 (*d*, 1H, J = 2 Hz, C-2′), 7 82 (*d*, 1H, J = 9 Hz, C-4), MS, *m/z* 216 $[M]^+$, 201, 188, 173, 160, 159, 158, 157

Xanthotoxin Mp 145°, UV $\lambda_{max}^{CHCl_3}$ nm 252 268 (sh), 300, IR ν_{max}^{KBr} cm^{-1} 3100, 1685, 1575, 1400, 1328, 1290, 1150, 1100, 875, 815, 755, ^{1}H NMR ($CDCl_3$, 90 MHz) δ 4 29 (*s*, 3H, –OMe), 6 38 (*d*, 1H, J = 9 5 Hz, C-3), 6 82 (*d*, 1H, J = 2 Hz, C-3′), 7 36 (*s*, 1H, C-5), 7 68 (*d*, 1H, J = 2 Hz, C-2′), 7 76 (*d*, 1H, J = 9 5 Hz, C-4), MS, *m/z* 216 $[M]^+$, 201, 188, 173

Acknowledgements—Financial assistance from CST and UGC, India is gratefully acknowledged

REFERENCES

1 Dreyer, D L, Pickering, M V and Cohan, P (1972) *Phytochemistry* **11**, 705
2 Talpatra, S K, Chaudhuri, M K and Talpatra, B (1973) *Phytochemistry* **12**, 236
3 *Supplement to Glossary of Indian Medicinal Plants* (Chopra, R N, Chopra, I C and Varma, B S, eds) (1969) p 29 Publications and Information Directorate, New Delhi
4 Chakraborty, D P (1959) *J Sci Ind Res* **18B**, 90
5 Gupta, S R, Seshadri, T R, Sharma, C S and Sharma, N D (1979) *Planta Med* **36**, 95
6 Shukla, Shriram and Tiwari, R D (1971) *Indian J Chem* **2**, 287

7 Some, T O (1964) *J. Pharm Sci* **53**, 231.
8. Elmofty, A M. (1968) *Vitiligo and Psoralen*, pp. 1–221. Pergamon Press, Oxford
9 Murray, R D H, Mendez, J. and Brown, S A (1982) *The Natural Coumarins*, p 31 Wiley-Interscience, New York.
10 Abu-Mustafa, E. A., El-Bay, F K. A, El-Khrisy, E A M and Fayez, M B E. (1973) *J Heterocyc Chem* **10**, 443
11 Prakash, D, Raj, K, Kapil, R S and Popli, S. P (1978) *Phytochemistry* **17**, 1194
12 Elgamal, M H A., Elewa, N H., El-Khrisy, E A M and Duddeck, H (1979) *Phytochemistry* **18**, 139
13 Murray, R. D H, Mendez, J and Brown, S A (1982) *The Natural Coumarins*, p. 28. Wiley-Interscience, New York
14 Pinder, A R (1960) *The Chemistry of the Terpenes*, p 35 Wiley, New York
15 Patra, A, Mukhopadhyay, A K, Ghosh, A and Mitra, A K (1979) *Indian J. Chem* **17B**, 385
16. Garg, S K, Gupta, S R and Sharma, N D (1978) *Phytochemistry* **17**, 2135