Phytochemistry, 1977, Vol. 16, pp. 1991–1993. Pergamon Press. Printed in England.

WIKSTROSIN, A TRICOUMARIN FROM *WIKSTROEMIA VIRIDIFLORA**

SHEELA TANDON and R. P. RASTOGI
Central Drug Research Institute, Lucknow-226001, India

(*Received* 20 *May* 1977)

Key Word Index—*Wikstroemia viridiflora*; Thymelaeaceae; bicoumarin; daphnoretin; tricoumarin; wikstrosin; structural determination.

Abstract—In the polar fraction of the extract from *Wikstroemia viridiflora*, daphnoretin and a new coumarin, wikstrosin, were identified. Wikstrosin has been characterised by chemical and spectral methods as a tricoumarin, a new class not reported hitherto in nature.

INTRODUCTION

A previous communication [1] reported the isolation and characterisation of a new lignan, wikstromol, together with pinoresinol, matairesinol and arctigenin, from *Wikstroemia viridiflora*. This plant has shown potent anticancer activity. The present paper describes further work on other fractions which have resulted in the isolation of two coumarins, E and F.

RESULTS AND DISCUSSION

Substance E, $C_{19}H_{12}O_7$ (M^+ 352) identified as daphnoretin (**1**) [2] was the major constituent of the plant. Substance F was found to be a new coumarin and named wikstrosin. It was a colourless powder and analysed for $C_{27}H_{14}O_9$ which was confirmed by high resolution MS (M^+ 482.0632). It gave a yellow colour with dilute alkali and fluoresced in UV light. The close similarity of its UV (λ_{max} 237, 330 nm log ε 4.482, 4.483), IR and PMR spectral pattern with that of umbelliferone suggested the coumarin nature of wikstrosin. The functional groups in the molecule were confirmed by derivatisation which gave a diacetate, a dibutryl derivative and a diMe ether, indicating the presence of two phenolic OHs in the molecule. IR showed a strong band at 1290 cm^{-1} which has been ascribed in a daphnoretin to an aromatic ether linkage (C=C—O—Ar). Its PMR exhibited only 12 protons in the aromatic region from 6.1 to 8 ppm which could be unambigously assigned by analogy with umbelliferone and bicoumol [3] (Table 1).

The MS of wikstrosin showed ions at *m/e* 465 (M^+-17), 464 (M^+-18), 438 (M^+-44), 409 (M^+-44–29), 381 (M^+-44–29–28), 353 (M^+-44–29–56), 322 (M^+-$C_9H_4O_3$), 294 (M^+-$C_9H_4O_3$–28), 277 (M^+-$C_9H_4O_3$–28–17), 265 (M^+-$C_9H_5O_3$–56), 237 (M^+-$C_9H_5O_3$–83), 219 (M^+-$C_9H_5O_3$–83–18) 181 ($C_{13}H_9O$), 162 ($C_9H_6O_3$). The prominent ion at *m/e* 162.0302 ($C_9H_6O_3$) suggested umbelliferone as the basic unit in the molecule. In view of the molecular formula $C_{27}H_{14}O_9$, wikstrosin was evidently a trimer of umbelliferone.

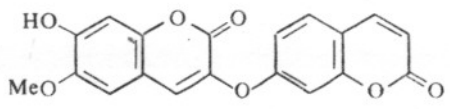
The oxidative degradations of wikstrosin with perchloric acid as well as chromium trioxide gave only umbelliferone in rather poor yield. In the latter case a faint spot (TLC) was also observed which was identical with bicoumol [3] (co-TLC, C_6H_6–EtOAc, 1:2) but it could not be isolated. Pyrolysis of the substance at *ca* 380° under reduced pressure proved to be most informative when umbelliferone was obtained in a yield of almost 70%. Thus, it was confirmed that trimerisation of umbelliferone takes place by loss of 4H atoms. Further, in view of the presence of two phenolic OHs in the molecule, the linking of the units must be through an ether bridge and a C—C bond. It should also be mentioned that similar experiments performed with daphnoretin and bicoumol under identical conditions resulted in the formation of umbelliferone and scopletin and umbelliferone respectively as the only products.

Considering the various alternatives in which 3 units of umbelliferone could be united subjected to the above requirements and involving linkages at C-3, C-6 and C-8 as indicated by PMR data, 6 structures are possible for wikstrosin. In this, the 3 units are linked as (i) C6-O-C7′, C3′-C8″ (ii) C6-O-C7′, C8′-C3″ (iii) C8-O-C7′, C6′-C3″ (iv) C8-O-C7′, C3′-C6″, (v) C3-O-C7′, C6′-C8″ and (vi) C3-O-C7′, C8′-C6″. The various links shown in these structures would be evident by **2a** and **2b** which denotes (v) and (vi).

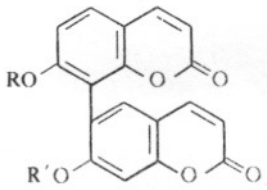
Table 1. PMR data (in ppm) of wikstrosin, bicoumol and umbelliferone

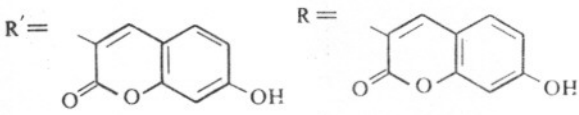
Assignment	Wikstrosin	Bicoumol	Umbelliferone
C-3′, 3″	6.1, 6.3 2H, each *d*, *J* = 9.5 Hz	6.31, 6.18 2H, each *d*, *J* = 9.5 Hz	6.1 1H, *d*, *J* = 9.5 Hz
C-4, 4′, 4″	7.91, 7.98 2H, each *d*, *J* = 9.5 Hz 7.5 1H, *s*	7.91, 7.97 2H, each *d*, *J* = 9.5 Hz	7.83 1H, *d*, *J* = 9.5 Hz
C-5, 5′, 5″	7.46 1H, *s* 7.65, 7.7 *J* = 8 Hz	7.54 1H, *d*, *J* = 8 Hz 7.48 1H, 4*S*	7.43 1H, *d*, *J* = 8 Hz
C-6, 6′	6.85, 7.01 2H, *dd*, *J* = 8, 2.5 Hz	6.94 1H, *d*, *J* = 8 Hz	6.73 1H, *dd*, *J* = 8, 2 Hz
C-8, 8″	7.1 2H, *br. s*	6.6 1H, *s*	6.63 1H, *s*

* CDRI Communication No. 2238.



1





2 a 2 b

The structures (i) and (ii) which have an esculin chromophore, were ruled out on the basis of the UV spectrum of wikstrosin which did not show any absorption at a longer wave length than 330 nm. A perusal of remaining structures would show that structures (iii) and (iv) have a 3-phenyl coumarin moiety whereas (v) and (vi) contain a biphenyl chromophore, etither of which could be confirmed by the physical data.

The three C-4,4′ and 4″ protons in wikstrosin showed almost identical chemical shift values (7.95 ppm) which ruled out the 3-phenyl coumarin unit (iii and iv) because in such structures the adjacent C-4 proton would appear at a higher field due to the phenyl ring current effect.

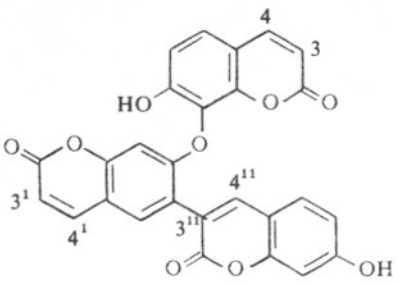
On the other hand, a biphenyl moiety in wikstrosin became evident from its optical activity. A perusal of the optical activities in phenyl coumarins and bicoumarins showed that optical activity has been reported [4] only in the case of kotanin and desmethyl-kotanin (+31.5° and −13.3° respectively) which was due to the presence of a stable rotamer because of restricted rotation in the biphenyl system. A high optical rotation (−82.0°) in wikstrosin clearly shows the presence of a stable rotamer. Hence, the structure of wikstrosin is **2a** or **2b** which is comprised of a basic bicoumol unit with an OH group and an ether bridge linking the third umbelliferone unit.

These structures are in full agreement with the PMR assignments and are also supported by the fact that bicoumol was one of the chromic acid oxidation products of wikstrosin.

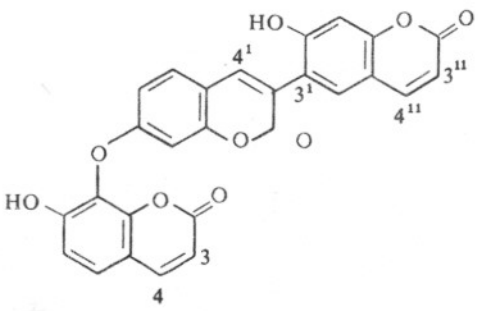
Although coumarins are the most exhaustively studied class of naturally-occurring heterocycles, the bicoumarins are comparatively new and only a dozen have been identified so far. Dicoumarol [5], the first isolated in 1941, has two 4-hydroxy coumarin units linked at C-3,3′ through a methylene bridge. Daphnoretin [2], was found to be another type wherein two coumarin units were linked by an ether bridge. A third type, matsukazelactone [6], isolated in 1964, contained a C—C linkage. Other members of this class isolated during the last decade are bicoumol [3], thamnosin [7], lasiocephalin [8], kotanin [4], phebalin, candicanin [10], euphorbetin [11], isoeuphorbetin [12] and edgeworthin [13]. The isolation and characterization of wikstrosin from *W. viridiflora* records the first member of the new class—tricoumarin, from a Thymelaeaceaeous plant.

EXPERIMENTAL

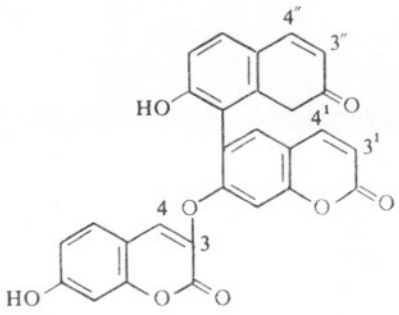
Mp's are uncorr. PMR spectra were recorded in DMSO-d_6 unless stated otherwise, with TMS as internal standard. The TLC values are for Si gel: $FeCl_3$–$K_3Fe(CN)_6$ spray regent. The



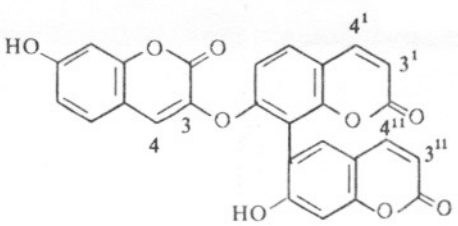
(iii) C-8—O—C—7′, C-6′—C-3″



(iv) C-8—O—C-7′, C-3′—C-6″



(v) C-3—O—O—C-7′, C-6′—C-8″



(vi) C-3 – O – C-7′, C-8′—C-6″

EtOH extract of the plant (3 kg) was macerated successively with hexane and EtOAc to yield hexane-soluble (23 g) and EtOAc-soluble (57 g) fractions. The latter fraction was chromatographed over hyflosupercel (300 g) and C_6H_6 (17 g), EtOAc (34 g) and MeOH (3 g) eluates were collected. The EtOAc eluate residue was rechromatographed over Si gel (1 kg) and 120 fractions (250 ml each) were collected using C_6H_6 containing increasing amounts of MeOH. The C_6H_6–MeOH (24:1) fractions (43–48) on crystallization from $CHCl_3$–MeOH yielded substance E (0.585 g). The subsequent fractions (49–58) gave substance F which was obtained as colourless powder (0.25 g) from MeOH.

Substance E (*daphnoretin*). Pale yellow needles, mp 243–47° decomp. It gave a yellow colour in alkali and showed white fluorescence in UV light; R_f 0.5 (C_6H_6–EtOAc, 1:1). λ_{max} (EtOH): 228, 265, 325, 343 nm (log ε 4.18, 3.86, 4.28, 4.31). ν_{max} (KBr): 3650 (OH) 1720 (unsaturated α-pyrone) 1613, 1592, 1481 (aromatic), 1282 (—C=C—O—), 1242, 1220, 1136, 1087, 1026, 917, 870, 850, 770, 738 cm^{-1}. PMR ppm: 3.82 (3H, *s*, —OMe), 6.3 (1H, *d*, J = 9.5 Hz, C-3′), 6.85–7.57 (5H, *m*, aromatic), 7.8 (1H, *s*, C-4), 8 (1H, *d*, J = 9.5 Hz, C-4′). MS *m/e*: 352 (M^+), 337, 324, 323, 322, 310, 304, 295, 281, 180, 179, 176, 173, 164, 162, 135, 134, 120, 119, 117. Found: C, 64.8; H, 3.3 $C_{19}H_{12}O_7$ requires C, 64.89; H, 3.36 percent. The *acetyl derivative* crystallised from $CHCl_3$, mp 247°. ν_{max} (KBr): 1763 cm^{-1} (phenolic acetate). PMR ppm: 2.36 (3H, *s*, OCOMe), 3.82 (3H, *s*, OMe), 6.38 (1H, *d*, J = 9.5 Hz, C-3), 7.08–7.8 (5H, aromatic), 7.9 (1H, *s*, C-4′) and 8.05 (1H, *d*, J = 9.5 Hz, C-4). MS *m/e*: 394 (M^+). The *methyl ether* was obtained as colourless needles from MeOH mp 228–31°. λ_{max}^{EtOH}: 227, 262, 324, 342 nm (log ε 4.30, 3.94, 4.32, 4.34). PMR ppm: 3.82, (3H, *s*, —OMe), 3.9 (3H, *s*, OMe). MS *m/e*: 366 (M^+).

Substance F (*wikstrosin*). Colourless powder, mp 318–320° decomp., $[\alpha]_D$ −82.3° (*c* 0.42, Py). It gave a yellow colouration with alkali and showed white fluorescence in UV light. R_f 0.28 (C_6H_6–EtOAc, 1:1) λ_{max}^{EtOH}: 237, 330 nm (log ε 4.482, 4.483), ν_{max}^{KBr}: 3274 (OH, 1734, 1700 (unsaturated α-pyrone), 1603, 1600, 1527 (aromatic), 1290 (—C=C—O—), 1418, 1389, 1325, 1250, 1143, 1099, 1047, 1005, 858, 844 cm^{-1}. PMR ppm: 6.1 and 6.3 (2H, each *d*, J = 9.5 Hz, C-3′, 3″), 6.9, 6.95 (2H, each *dd*, J = 2.5, 8 Hz, C-6, 6′), 7.1 (2H, *s*, C-8, 8″), 7.46 (1H, *s*, C-5″), 7.65 and 7.7 (2H, each *d*, J = 8 Hz, C-5, 5′), 7.95 (1H, *s*, C-4), 7.91 and 7.98 (2H, each *d*, J = 9.5 Hz, C-4′, 4″). MS *m/e*: 482.0632 (M^+, 75, $C_{27}H_{14}O_9$), 465 (24), 464 (33), 438.0710 (100, $C_{26}H_{14}O_7$), 409.0959 (10), 381 (5), 353 (4), 322 (4), 294.0567 (10, $C_{17}H_{10}O_5$), 277.0494 (8, $C_{17}H_9O_4$), 265.0521 (53, $C_{16}H_9O_4$), 237 (11), 219.0406 (7, $C_{15}H_7O_2$), 209 (9), 181·0641 (9, $C_{13}H_9O$), 162.0302 (48, $C_9H_6O_3$), 134.0347 (35), 105 (1). Found: C, 67.00; H, 314. $C_{27}H_{14}O_9$ requires C, 67.1; H 2.9%.

Acetylation of F. The substance (30 mg) was reacted overnight in Py (0.5 ml) with Ac_2O (0.5 ml). After working up, the residue was crystallized from EtOH (28 mg), mp 225–228°, ν_{max}^{KBr}: 1776 cm^{-1} (phenolic acetate), PMR (acetone-d_6) ppm: 2.01, 2·05 (3H each, *s*, OCOMe), 6.3, 6.4 (2H, each *d*, J = 10 Hz, C-3′, 3″), 6.9–7.9 (10H, *m*, aromatic). MS *m/e*: (M^+ absent), 462, 436, 408, 379, 320, 376, 252, 248, 224, 162. Found: C, 65.62; H, 3.26. $C_{31}H_{18}O_{11}$ requires C, 65.73; H, 3.1%.

Butrylation of F. Substance (30 mg) in Py (0.5 ml) was reacted with *n*-$BuCO_2O$ (0.5 ml) overnight at room temp. The reaction mixture was freed of solvent and the residue macerated with hexane to remove excess reagent. The insoluble material crystallized from EtOH as colourless needles (35 mg), 200° decomp. R_f 0.54 (C_6H_6–EtOAc, 1:1). PMR ppm: 1.8–2 (6H, *m*, 2-$CH_2\underline{CH_3}$), 3.35 (8H, *br. s*, 2-$C\underline{H_2}C\underline{H_2}CO$—), 6.3, 6.4 (2H, each *d*, J = 9.5 Hz, C-3′, 3″), 7.16 (2H, *s*, C-8, 8″), 7.3 and 7.31 (2H, each *d*, J = 8 Hz, C-6, 6′), 7.59 (1H, *s*, C-5″), 7.8 and 7.82 (2H, each *d*, J = 8 Hz, C-5, 5′), 7.91 (1H, *s*, C-4), 8.08, 8.1 (2H, each *d*, J = 9.5 Hz, C-4′, 4″).

Methylation of F. Substance F (45 mg), Me_2SO_4 (0.5 ml) and anhydrous K_2CO_3 (1.5 g) were refluxed in dry Me_2CO (25 ml) for 8 hr in an inert atmospher. The reaction mixture was concd, diluted with H_2O and filtered. The residue crystallized from MeOH as colourless needles (35 mg), mp 188–189°. λ_{max}^{EtOH}: 221 and 336 nm. PMR ($CDCl_3$) ppm: 3.83 (6H, *s*, 2 OMe), 6.2 and 6.3 (2H, each *d*, J = 10 Hz), 6.9–7.5 (9H, *m*, aromatic), 7.99 (1H, *s*, C-4), 7.6 and 7.78 (2H, each *d*, J = 10 Hz, C-4, 4″). MS *m/e*: 510 (M^+), 496, 478, 371, 352, 337, 291, 263, 249, 223, 221, 163, 139, 134. Found: C, 68·4; H, 2·89. $C_{29}H_{18}O_9$ requires C, 68.3, H, 2.8 percent.

Pyrolysis of F. The substance (26 mg) was pyrolyzed in a sublimation tube at 378–80° for 15 min at 20 mm. The sublimate (18 mg) showed the presence of only one spot on TLC and crystallized from EtOH as colourless needles, mp 226°, R_f 0.75 (C_6H_6–EtOAc). λ_{max}^{EtOH}: 220 and 325 nm. ν_{max}^{KBr}: 3200, 1695, 1613, 1557, 1520, 1490, 1429, 1325, 1250, 1143, 1111, 995, 840 cm^{-1}. PMR ppm: 6.1 (1H, *d*, J = 9.5 Hz, C-3), 6.63 (1H, *s*, C-8), 6.73 (1H, *d*, J = 8 Hz, C-6), 7.43 (1H, *d*, J = 8 Hz, C-5), 7.83 (1H, *d*, J = 9.5 Hz, C-4). MS *m/e*: 162 (M^+).

Acknowledgements—The authors thank Mr. Edward Samson for technical assistance during this work. They gratefully acknowledge that the plant material was collected by financial support under a NIH scheme (Grant No. PL-480, 134304). They also thank Prof. Dr. F. Bohlmann, Berlin, for the high resolution MS of wikstrosin reported in this communication and to Prof. Dr. R. Tschesche, Bonn, West Germany, for a sample of daphnoretin.

REFERENCES

1. Tandon, S. and Rastogi, R. P. (1976) *Phytochemistry* **15**, 1789.
2. Tschesche, R., Schaelt, U. and Regler, G. (1963) *Ann. Chem.* **662**, 113.
3. Spencer, R. R., Wilt, S. C., Ludin, R. E. and Bickoff, E. M. (1967) *J. Agric. Food Chem.* **15**, 536.
4. Buchi, G., Klaubert, D. H., Shank, R. C., Weinberg, S. M. and Wagon, G. M. (1971) *J. Org. Chem.* **36**, 1143.
5. Stahmann, M. A., Hubner, C. F. and Link, K. P. (1941) *J. Biol. Chem.* **138**, 513.
6. Miyazaki, T., Mihashi, S. and Okabayashi, K. (1964) *Chem. Pharm. Bull. (Tokyo)* **12**, 1232.
7. Kutney, J. P., Inaba, T. and Dreyer, D. L. (1968) *J. Am. Chem. Soc.* **90**, 813.
8. Bhattacharya, A. K. and Das, S. C. (1971) *Chem. Ind. (London)* 885.
9. Brown, K., Cambie, R. C. and Hall, D. (1971) *Chem. Ind. (London)* 1020.
10. Bandopadhyay, M., Malik, S. B. and Seshadri, T. R. (1971) *Tetrahedron Letters* 4221.
11. Dutta, P. K., Banerjee, D., and Dutta, N. L. (1972) *Tetrahedron Letters* 601.
12. Dutta, P. K., Banerjee, D. and Dutta, N. L. (1973) *Ind. J. Chem.* **11**, 831.
13. Majumdar, P. L., Sengupta, G. C., Dinda, B. N. and Chaterjee, A. (1974) *Phytochemistry* **13**, 1929.