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STRUCTURE OF BISOSTHENON, A NOVEL DIMERIC COUMARIN FROM *CITRUS* PLANTS[1)]

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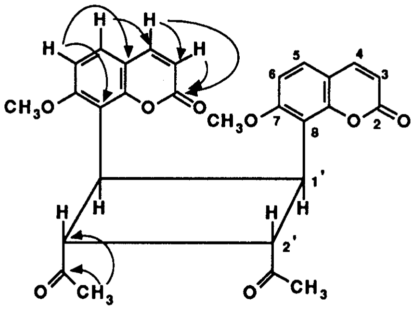
A new dimeric coumarin, named bisosthenon (**1**), was isolated from the roots of some *Citrus* plants. The structure **1**, corresponding to a head-to-head dimer of osthenon (**4**), previously obtained from *Citrus* plants, was elucidated by spectrometric and X-ray analysis. The photodimerization of **4** was also studied.

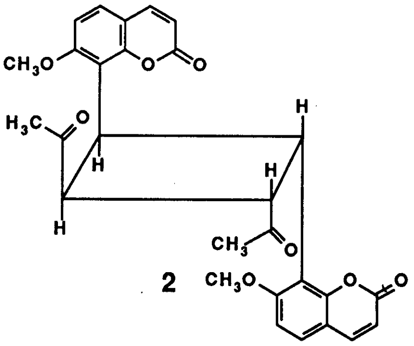
KEYWORDS coumarin; Rutaceae; *Citrus*; bisosthenon; osthenon; photodimerization; dimer; cyclobutane; X-ray

Our phytochemical studies of *Citrus* plants were continued to characterize many kinds of new coumarins and acridone alkaloids as well as known components.[2)] We here report on the structure of a novel dimeric coumarin, bisosthenon (**1**), from roots of some *Citrus* plants: *C. funadoko* Hort. ex Y. Tanaka (funadoko); several hybrid seedlings resulting from crosses of *C. flaviculpus* Hort. ex Tanaka (ogonkan) X *C. Tamurana* Hort. ex Tanaka (hyuganatsu) and [*C. unshiu* Marc. (miyagawa-wase) X *C. sinensis* Osbec. (torobita orange)] (kiyomi) X *C. iyo* Hort. ex Tanaka (iyo). As reported previously,[2)] the acetone extracts of the dried roots of the plants were subjected successively to silica gel column, preparative centrifugally accelerated thin-layer, and preparative thin-layer chromatographies to yield bisosthenon (**1**) in addition to many other coumarins and/or acridones.

Bisosthenon (**1**) was isolated as colorless prisms, mp 234 - 237°C, UV λ_{max} (MeOH) 253, 260, 317 nm; IR ν_{max} ($CHCl_3$) 1725, 1720, 1605 cm^{-1}; $C_{28}H_{24}O_8$ [m/z 488.1451 M^+, found]; 488.1469, calcd.]. In the ^1H- and ^{13}C-NMR spectra, the number of signals observed (Table I) was half of that expected, suggesting that bisosthenon (1) had a completely symmetrical structure. In the ^1H-NMR spectrum, a methoxy signal appeared at δ 3.60, and two pairs of AB-type doublets at δ 6.09 and 7.47 (each 1H, J = 9.4 Hz) and δ 7.11 and 6.52 (each 1H, J = 8.7 Hz) were assignable to H-3, 4, 5, and 6, respectively, and from the analysis of H-C COSY spectrum, signals of carbons bearing these protons were located at δ 112.54, 143.68, 126.98, and 106.69, respectively. These spectral data, coupled with the results of a long-range H-C COSY spectrum, shown by arrows in formula **1**, indicated the presence of the 7-methoxy-8-substituted coumarin nucleus in bisosthenon molecule. The presence of an acetyl moiety in **1** was deduced from the appearence of IR absorption at ν_{max} 1725 cm^{-1}, a three-proton singlet at δ_H 2.27, and a carbonyl carbon singlet at δ_C 208.27. The remaining protons at δ_H 4.93 (1H, d, J = 6.7 Hz) and 4.82 (1H, br s) were coupled to each other and carbons bearing these protons appeared at δ_C 35.15 and 51.04, respectively. These data were suggestive of a 1,2,3,4-symmetrically-substituted cyclobutane ring system.[3,4)] The ^1H-NMR signal pattern of bisosthenon was similar to that of osthenon (**4**)[2)] isolated previously from some *Citrus* plants, except for H-1' and H-2' signals. The EI-MS spectrum of bisosthenon exhibited a characteristic fragment peak at m/z 244 which corresponded to a half of the molecule, $C_{14}H_{12}O_4$, and also showed peaks at m/z 229, 213 (base peak), and 201, associated, respectively, with loss of a methyl, a methoxy, and an acetyl radical from half of a molecular ion (m/z 244). From the foregoing spectroscopic data, it was concluded that bisosthenon was either the head-to-head (a) or head-to-tail (b) dimer of osthenon (4). According to the mass spectrometric analysis reported previously,[3,4] absence of mass fragment peaks at m/z 376 and 112 even in enlarged mass spectrum of bisosthenon pointed to a head-to-tail dimeric structure b, in contrast to the actual head-to-head structure a determined by X-ray crystallographic analysis (*vide infra*).



1



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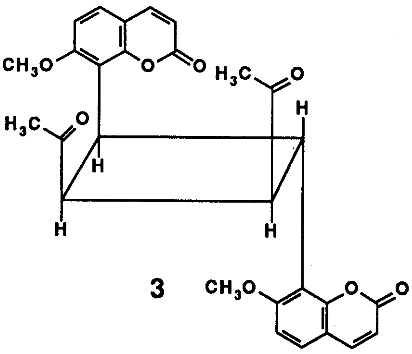

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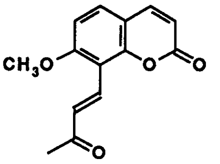

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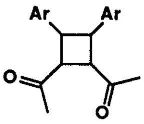
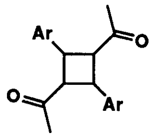


a



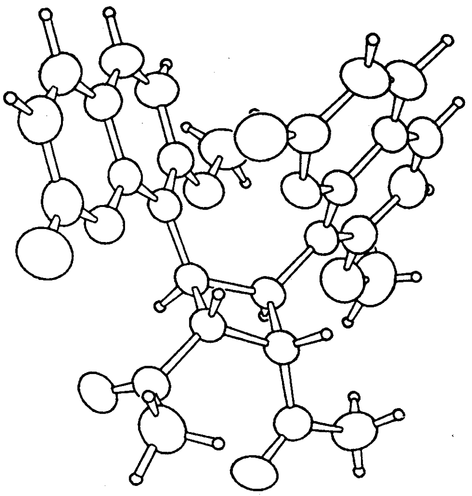
b

Table I. The ^1H- and ^{13}C-NMR Spectra of 1 and 2

NO.	1 δ_H	1 δ_C	2 δ_H	2 δ_C
2	---	160.43	---	161.80
3	6.09(d,9.4)	112.54	6.15(d,9.5)	113.14
4	7.47(d,9.4)	143.68	7.56(d,9.5)	143.66
4a	---	112.54	---	112.95
5	7.11(d,8.7)	126.98	7.33(d,8.5)	127.65
6	6.52(d,8.7)	106.69	6.89(d,8.5)	108.19
7	---	153.25	---	152.95
8	---	115.93	---	116.63
8a	---	160.27	---	160.51
OCH_3	3.60(s)	55.35	3.96(s)	56.03
1'	4.93(d,6.7)	35.15	4.95(m*)	33.71
2'	4.82(br.)	51.04	4.07(m*)	49.34
3'	---	208.27	---	207.66
CH_3	2.27(s)	28.65	2.13(s)	27.48

Taken in $CDCl_3$. m*: doublet-like signal. Figures in parentheses are J (Hz).

Fig 1. Structure and Solid-state Conformation of Bisosthenon (1)



Single-crystal X-ray analysis established the complete structure and relative stereochemistry of bisosthenon. Crystal data: $C_{28}H_{24}O_8$, M = 488.50, triclinic, space group $P1(C_i^1)$-No.2, a = 11.003(2), b = 13.721(2), c = 8.707(2) Å, α = 106.60(2), β = 106.41(2), γ = 73.03(2)°, V = 1176(1) $Å^3$, Z = 2, $D_{calcd.}$ = 1.379 g cm^{-3}. The crystal structure was solved by direct methods (MULTAN11/82). Full-matrix least-squares refinement of atomic positional and thermal parameters (anisotropic C, O; isotropic H) converged to R = 0.046

(R_w = 0.066) over 3733 reflections with $I > 3.0\,\sigma(I)$ recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-K_α radiation, incident-beam graphite monochromator; ω-2θ scans, θ_{max} = 75°). A view of the structure is provided in the Fig. 1.

Next, we carried out the synthesis of **1**. Photodimerization[5)] of **4**[6)] in a solid-state using a high-pressure Hg-lamp and a Pyrex glass filter for 142 hours at room temperature yielded three dimeric products (A, B, and C in 16, 11, and 2% yields, respectively) accompanied by a 9% *cis*-isomer and 35% recovery of **4**.[7)] The similarity of the MS fragmentation patterns of these three dimeric products indicated that they were the stereoisomers. Spectral comparison (IR, MS, ^{1}H-NMR) verified that the most polar product A was identical with natural bisosthenon (**1**). Detailed NMR studies[8)] indicated that the product B had unsymmetrical structure **3**. The remaining minor product C gave symmetrical ^{1}H- and ^{13}C-NMR signal patterns (Table I) which overall were similar to those of **1** after allowing for some chemical shift differences. In the NOE experiments of C, irradiation of the signal at δ 4.95 (H-1') gave 7% and 3% enhancements of the respective signals at δ 4.07 (H-2') and 2.13 (acetyl methyl proton), whereas irradiation of the signal at 4.07 (H-2') caused 8% and 4% area increases of the signal at δ 4.95 (H-1') and 2.13 (acetyl), respectively.[9)] These results indicated that C contained the *trans*, *cis*, *trans*-cyclobutane ring structure **2**.[10)]

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6) Osthenon (**4**) was synthesized from commercially available umbelliferone by succssesive treatments as follows: 1) I_2, KI in NH_4OH, 2) MeI, K_2CO_3 in Me_2CO, 3) $MeCOCH{=}CH_2$, $Pd(OAc)_2$, Me_3N, TBAB in DMF.
7) Photodimerization of **4** in isopropyl alcohol gave product A [bisosthenon (**1**)] as a sole product (yield 15%, *cis*-osthenon: 35%, recovery of **4**: 8%).
8) **3**: ^{1}H-NMR (50°C, $CDCl_3$) δ: 7.48 (1H, d, J = 9.4 Hz), 7.45 (1H, d, J = 9.4 Hz), 7.12 (1H, d, J = 8.7 Hz), 7.05 (1H, d, J = 8.7 Hz), 6.46 (1H, br.), 6.42 (1H, d, J = 8.7 Hz), 6.15 (1H, br.), 6.11 (1H, d, J = 9.0 Hz), 5.69 (1H, t, J = 10.4 Hz), 5.42 (1H, t, J = 10.4 Hz), 4.49 (1H, t, J = 10.4 Hz), 3.75 (1H, dd, J = 9.4 Hz), 3.55 (6H, s), 2.45 (3H, s), 1.77 (3H, s).
9) In the NOE experiments of bisosthenon (**1**) having a *cis*, *trans*, *cis*-cyclobutane ring system, a strong negative NOE enhancement was observed at the signal of δ 4.82 (H-2') on irradiation of the signal at δ 4.93 (H-1'). Irradiation of the signal at δ 4.82 (H-2') also showed a strong (-)-NOE enhancement of the signal at δ 4.93 (H-1').
10) The alternative structure having a *cis*, *cis*, *cis*-cyclobutane ring system may be excluded due to the severe steric strain which would be present.

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