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STRUCTURE REVISION OF A FURANOCOUMARIN FROM *DORSTENIA CONTRAJERVA*

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Key Word Index—*Dorstenia Contrajerva*; Moraceae; furanocoumarins; structure revision; crystal structure.

Abstract—Two furanocoumarins have been isolated from *Dorstenia contrajerva* L.: bergapten and 4-[[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butyl]oxy]-7*H*-furo[3,2-g][1]benzopyran-7-one. The structures were established by UV, NMR and mass spectrometry. The structure of the second compound, an unusual furanocoumarin, was confirmed by X-ray analysis. This substance has already been isolated from *D. contrajerva*, but the structure was incorrectly elucidated.

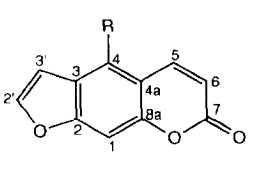
INTRODUCTION

During the course of our systematic search for new compounds of natural origin, Panamanian medicinal plants have been screened. *Dorstenia contrajerva* L. is a herbaceous plant reported to be used by the Kuna Indians of Panama as a cold remedy [1] and for the treatment of snakebites and muscle aches [2]. In the screening for biological activity, the dichloromethane extract did not show antifungal properties but exhibited a slight larvicidal activity against *Aedes aegypti*, the vector of yellow fever, [3] and antimicrobial activity against *Bacillus subtilis* [4]. But in fact, the interest devoted to this plant was due to the HPLC-UV analysis of the extract. The chromatogram of the dichloromethane extract showed a simple feature with only two main peaks having typical UV spectra of furanocoumarins. These two compounds were isolated and their structures determined by spectral methods as bergapten (**1**) and 4-[[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butyl]oxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one (**2**). X-Ray analysis of **2** confirmed its structure and also showed the presence of a racemic mixture. Another furanocoumarin with a monoterpenoid side chain (**3**) has previously been described as a major constituent of *D. contrajerva* [5]. However, this product was not detected in our plant sample, but comparison of the data of both compounds indicated a full correlation. Thus, the structure of furanocoumarin **3** was revised to that of **2**.

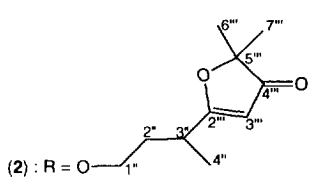
RESULTS AND DISCUSSION

Separation of the dichloromethane extract of *D. contrajerva* on a silica gel open column yielded **1** and **2** after crystallization. The compounds were purified by recrystallization to give needles and small prisms, respectively. Compound **1** was rapidly identified from ^1H and ^{13}C NMR data and by comparison with an authentic sample as bergapten.

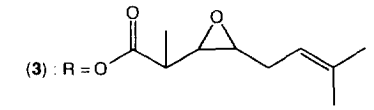
The structure of the side chain of the other furanocoumarin (**2**) was elucidated by ^1H and ^{13}C NMR, 2D-COSY NMR, mass spectrometry and confirmed by X-ray crystallography, which fixed the conformational



(**1**) : R = OMe



(**2**) : R = O—



(**3**) : R = O

‖Author to whom correspondence should be addressed.

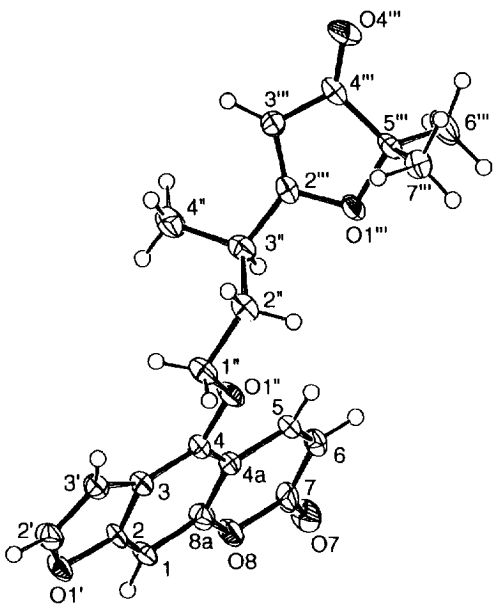


Fig. 1. Molecular formula of **2** as determined by X-ray analysis.

form of **2** (Fig. 1 gives the ORTEP plot of **2**). It should be noted that the X-ray analysis of **2** showed the presence of a racemic mixture and this was confirmed by the determination of the $[\alpha]_D$ value as $-3.3°$, a value quite near to 0.

This compound was already known from *Dorstenia cayapiaa* [6] and all the NMR data were in complete agreement with those indicated in this reference. Recently, **2** was also isolated from the rhizomes of another species of *Dorstenia*: *D. brasiliensis* [7], but an $[\alpha]_D$ value of $+1.4°$ was reported.

In the EI mass spectrum of **2**, the molecule (*m/z* 368) is fragmented into two parts: *m/z* 202 and 167. The ions at *m/z* 202, 174 and 145 can be attributed unequivocally to the furanocoumarin moiety [8]. The other peaks at *m/z* 139 and 111, together with the signal at *m/z* 167, belong to the side chain of the molecule. These values are explained by the loss of two C_2H_4 groups successively leaving the 4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl cation [7]. The next step of the fragmentation is probably the loss of an isopropyl group from the position 5''', giving a unit with *m/z* 69 containing the two oxygen atoms.

A previous study of *D. contrajerva* [5], collected at the Fairchild Tropical Garden in Miami, FL, yielded another furanocoumarin (**3**), with an epoxide-containing monoterpenoid side chain (5-EDOP, 5-[3,4-epoxy-2,7-dimethyl-6,7-octenoyl]psoralen). This compound, an isomer (*m/z* 368) of **2**, was isolated by preparative TLC from the chloroform-soluble fraction of the methanol extract. Surprisingly, even using the most modern analytical techniques (including LC-TSP-MS), this furanocoumarin was not detected in the dichloromethane extract or the methanol extract of our plant sample. By comparing the physical and spectral data of **3** (mp, UV, MS, ^{1}H NMR) mentioned in the literature with the original data of **2**, we found that everything was identical except one difference in the ^{1}H NMR spectrum. Consultation of the original ^{1}H and ^{13}C NMR spectra of **3** showed that they were strictly the same as those of **2**. As a result, we ascertain that the structure of the furanocoumarin described by Swain *et al.* [5] is not correct and should be replaced by the structure of **2**. The mistake was due to an error in interpreting the ^{1}H NMR spectrum and to the low sensitivity of the ^{13}C NMR spectrum.

Methanol and dichloromethane extracts were tested in our different benchtop screening assays. The dichloromethane extract exhibited a slight larvicidal activity and antibacterial properties. The larvicidal effect was tested against larvae of *Aedes aegypti* in a routine test [3] and the dichloromethane extract was active (100% of larvae killed in 24 hr) at a concentration of 125 ppm. All the fractions were also tested. Three of them were active, but less than the total extract. Both **1** and **2** were inactive. The antibacterial test against *Bacillus subtilis* was performed by direct bioautography [4]. The dichloromethane extract was active with a 30 μg sample deposited on the TLC plate. However, the active constituents exhibited tailing on TLC and were not identified in this work.

Furthermore, geiparvarin, a closely related 7-substituted coumarin with the same monoterpenoid side chain, was demonstrated to inhibit *in vitro* human carcinoma of the nasopharynx [9]. Consequently, **2** was tested at the NCI for its potential antitumour properties. But our compound did not show significant activity on the different strains of cancer cells tested.

EXPERIMENTAL

General. Mps: uncorr., measured on a Mettler FP 80/82 apparatus. EI-mass spectra were recorded on a Finnigan MAT TSQ 700 triple-stage quadrupole MS instrument. ^{1}H and ^{13}C NMR spectra were recorded at 200.03 and 50.30 MHz, respectively, in $CDCl_3$ with TMS as int. standard.

Plant material. The whole plant of *Dorstenia contrajerva* L. was collected in July 1992 in Parque Soberania, Canal Area, Panama and identified by Prof. Mireya D. Correa (Smithsonian Tropical Research Institute, Panama). A voucher specimen is deposited at the Herbarium of the University of Panama.

Extraction and isolation. The powder of the whole plant (230 g) was extracted successively with CH_2Cl_2 and MeOH (3 × 1 l of each). The CH_2Cl_2 extract was fractionated on a silica gel Si 60 open column (60 × 5 cm) using a step-gradient of petrol–EtOAc (4:1–1:2), EtOAc and finally MeOH to give 11 fractions (A1–A11). Bergapten (**1**) crystallized from fr. A7 and crystals of **2** were obtained from fr. A10. Both compounds were purified by recrystallization in a mixture of *n*-hexane and EtOAc.

Bergapten (**1**). Mp 190–191°. UV λ_{max}^{MeOH} nm (log ε): 225 (4.03), 251 (4.06), 259 (4.06), 267 (4.05), 308 (4.05). ^{1}H and

^{13}C NMR data in accord with ref. [7]. EI-MS: *m/z* 216 $[M]^+$.

4-[[3-(4,5-*dihydro*-5,5-*dimethyl*-4-*oxo*-2-*furanyl*)-*butyl*]*oxy*]-7H-*furo*[3,2-g][1]*benzopyran*-7-*one* (**2**). Mp 141–142°. UV λ_{max}^{MeOH} nm (log ε): 222 (4.03), 249 (4.18), 260 (4.17), 267 (4.17), 309 (4.07). $[\alpha]_D$ − 3.3° ($CHCl_3$; *c* 0.5). ^{1}H NMR (200.03 MHz, $CDCl_3$): δ 1.33 (3H, *d*, *J* = 7 Hz, H-4″), 1.31 (3H, *s*, H-6‴), 1.33 (3H, *s*, H-7‴), 2.14 (2H, *m*, H-2″), 2.98 (1H, *q*, *J* = 7 Hz, H-3″), 4.46 (2H, *m*, H-1″), 5.38 (1H, *s*, H-3‴), 6.23 (1H, *d*, *J* = 10 Hz, H-6), 6.86 (1H, *d*, *J* = 2.4 Hz, H-3′), 7.08 (1H, *s*, H-1), 7.56 (1H, *d*, *J* = 2.4 Hz, H-2′), 8.08 (1H, *d*, *J* = 10 Hz, H-5). ^{13}C NMR (50.30 MHz, $CDCl_3$): δ 17.8 (C-4″), 22.8 (C-6‴,C-7‴), 32.3 (C-3″), 34.2 (C-2″), 70.1 (C-1″), 88.5 (C-5‴), 94.1 (C-1), 100.1 (C-3‴), 104.7 (C-3′), 106.6 (C-4a), 112.7 (C-6), 113.3 (C-3), 138.9 (C-5), 145.0 (C-2′), 148.4 (C-8a), 152.5 (C-4), 158.1 (C-2), 160.9 (C-7), 194.0 (C-2‴), 207.1 (C-4‴). EI-MS: *m/z* 368 $[M]^+$ (100), 202 (17), 174 (14), 167 (33), 139 (98), 111 (8), 83 (12), 69 (13).

X-Ray analysis. Suitable crystals of **2** were grown from hexane–EtOAc as prisms. Intensity data were collected at − 80° on a Stoe AED2 4-circle diffractometer using MoK_α graphite monochromated radiation (λ = 0.7073 Å) with ω/2Θ scans in the 2Θ range 3–50°. The structure was solved by Direct Methods using the NRCVAX system [10], which was used for all further calculations. The H-atoms were refined isotropically and the non-hydrogen atoms anisotropically using weighted full-matrix least-squares. Crystal data for **2**: $C_{21}H_{20}O_6$, triclinic, space group *P*1, *a* = 9.015 (3), *b* = 9.960 (3), *c* = 10.934 (3) Å, α = 76.88 (2), β = 68.99 (2), γ = 81.13 (2)°, *V* = 889.7 (5) Å^3, *Z* = 2. 1990 observed reflections [I > 3σ(I)], final *R* = 0.061, R_w = 0.087. Further details of the crystal structure investigation can be obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ (U.K.) on quoting the full journal citation.

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