Synthesis, Crystal Structure Determination and Pharmacological Activity of 7,8,3′,4′-Tetramethoxyisoflavone

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The 7,8,3′,4′-tetramethoxyisoflavone **2** was synthesized and its structure confirmed by X-ray crystallographic analysis. Pharmacological screening was carried out with this compound in order to assess its pharmacological profile. Isoflavone **2** possessed antiinflamatory activity in the carrageenan oedema test, with a dose-effect relationship comparable to that of hydroxylated flavonoids. It is noteworthy that **2** is one of the few isoflavones efficient as antiinflammatory with complete alkylation of hydroxyl groups.

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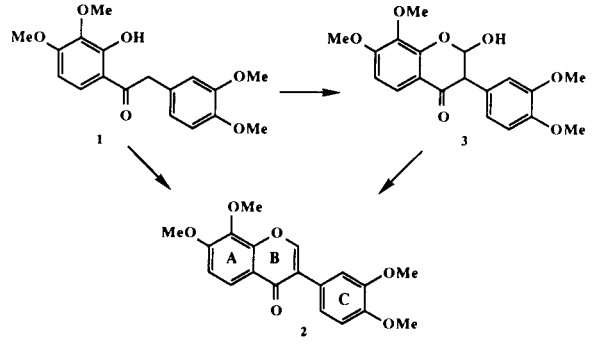
Isoflavones [1] are the most abundant subset of the flavonoid class of compounds which also includes pterocarpans, rotenoids, and coumestans. Structurally, isoflavones are highly substituted and oxygenated derivatives of 3-phenylchromanes. Much work on the biosynthesis of isoflavones from phenylpropyl precursors has been reported [2], but the complete pathway has not yet been defined. The highly oxygenated versions of isoflavones often have estrogenic activity and crude preparations of these compounds have also been used as fish narcotics, insecticides and antifungals for many years in Central and South America [3]. This wide range of biological properties has stimulated interest in the synthesis of natural and unnatural analogues of isoflavones.

As part of our program on the chemistry of heterocyclic systems as potential pharmacological agents, we reported [4] the isolation of 7,8,3′,4′-tetramethoxyisoflavone **2** by treatment of 2-hydroxy-3,4-dimethoxyphenyl 3,4-dimethoxybenzyl ketone **1** with formamide. In this paper we report an improved procedure for the synthesis of the isoflavone **2**. An X-ray analysis of **2** has been performed, confirming the structure originally predicted by chemical and spectroscopic methods. Finally, its pharmacological activity has also been tested and to our knowledge this is one of the few examples of isoflavones with antiinflamatory activity.

Results and Discussion.

Synthesis of isoflavones falls into two main categories (i) routes deriving from chalcone-based systems [5] and (ii) syntheses from benzoins precursors [3]. We have previously reported [4] a regioselective synthesis of deoxybenzoin **1** *via* Friedel-Crafts acylation of the 1,2,3-trimethoxybenzene with aluminium trichloride as catalyst. Therefore, we decided to prepare 7,8,3′,4′-tetramethoxyisoflavone **2** by formylation of the above mentioned deoxybenzoin **1**, being ethyl formate [6] and *N,N*-dimethylformamide dimethylacetal [7] the reagents of choice. Thus, cyclization of the ketone **1** with ethyl formate in the presence of sodium afforded the intermediate 2-hydroxy-7,8,3′,4′-tetramethoxyisoflavanone **3** (yield 50%), which was quantitatively dehydrated by means of acetic anhydride to the desired isoflavone **2**. It is also noteworthy that the hydroxy derivative **3** could be easily dehydrated when passed through a silica gel column and eluted with dichloromethane-ethyl acetate 95:5. In fact, tlc (dichloromethane-ethyl acetate 95:5 as eluent) of a sample of **3**, whose purity had been previously established by nmr, always showed two spots, one of R_F = 0.3 was the hydroxy derivative **3** and the other, R_F = 0.4, the isoflavone **2** (Scheme I).

Scheme I



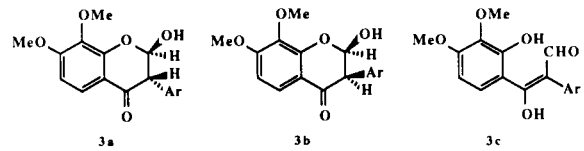
Interpretation of the nmr spectra of the hydroxy compound **3** revealed the presence of a mixture of diastereomeric hemiacetals. Although **3** can also exists in an open-chain keto form, the solvents used did not permit to observe appreciable amounts of this isomer. Another interesting aspect of the structure of **3** is the preference for the axial hydroxyl group in both diastereoisomers, related to the anomeric effect in carbohydrate chemistry [8,9]. In these hemiacetals the interaction of the aryl group at C-3 with the carbonyl group may allow a pseudoaxial or axial disposition for this substituent. The preferred conformation of the dihydropyrone ring might probably be a half-chair distorted toward the 1,2-diplanar (sofa) form, similar to the conformation of other flavanones in the solid state [10,11], and attributed to the presence of the keto group at C-4.

In fact, compound **3** showed in the pmr (250 MHz, deuteriochloroform) spectrum the existence of some duplicated peaks, in particular the doublet of the AX spin system for H-2 and H-3 protons. Thus, two distinct H-2 proton resonances were observed at δ 5.95 (J_{AX} = 3.4 Hz) and δ 5.91 (J_{AX} = 2.9 Hz), while the H-3 resonances occurred at δ 3.91 (J_{AX} = 3.4 Hz) and δ 4.12 (J_{AX} = 2.9 Hz) respectively. These assignments were confirmed by means of homonuclear spin-spin decoupling experiments. The lack of the aldehydic proton signal excluded a significant contribution by the open-chain keto form in this solvent. On the other hand, the magnitudes of the AX vicinal coupling constants indicated that the hydroxyl group was axial in both diastereomers, while the aryl group at C-3 was pseudoequatorially and pseudoaxially disposed in the major (*cis*) and minor (*trans*) isomers, respectively. The coupling for both isomers could be consistent with a sofa preferred conformation, similar to that found in the solid state on other flavanones. However, there remains too great an uncertainty in distinguishing the modest conformational differences between half-chair and sofa forms in solution.

We also observed the presence of both diasteromeric hemiacetals, but no open-chain keto form, in other solvents (DMSO-d_6, acetone-d_6, and in mixtures of them with carbon tetrachloride of varied composition). For instance, in deuteriochloroform the relative ratios by integration of the isomers **3a**, **3b**, and **3c** were 40:60:0, while in acetone-d_6 they were 30:70:0. This difference in the isomeric populations may be attributed to the ability of the solvent to stabilize one epimer over another.

The cmr spectrum (resonances were assigned on the basis of chemical shifts and DEPT measurements) of this compound supported this hemiacetal structure in solution and showed the resonances due to both interconverting isomers. The most characteristic signals were the four resonances for C-2 and C-3 carbons of the hemiacetals. The comparable ^{13}C resonances for the open-chain keto form could not be observed.

Figure I



3a 3b 3c

On the other hand, formylation of deoxybenzoin **1** also occurred upon treatment with *N,N*-dimethylformamide dimethylacetal in anhydrous benzene to give the isoflavone **2** in better yield (80%).

The X-ray structure determination of isoflavone **2** confirmed the identity of this compound. Figure II shows a stereoscopic view of the molecule and the atom labels [12]. The bond lengths and angles are given in Tables I and II, respectively and are in good agreement with the values reported for similar flavonoid and isoflavonoid structures [13].

Table I

Bond Lengths (Å) of Isoflavone 2

C(1)-C(2)	1.380(4)	C(9)-C(14)	1.393 (4)
C(1)-C(6)	1.403 (5)	C(10)-C(11)	1.373 (4)
C(1)-C(7)	1.486 (4)	C(11)-C(12)	1.394 (5)
C(2)-C(3)	1.387 (4)	C(12)-C(13)	1.385 (4)
C(3)-C(4)	1.372 (5)	C(12)-O(24)	1.363 (4)
C(4)-O(19)	1.366 (4)	C(13)-O(22)	1.373 (4)
C(5)-C(6)	1.373 (4)	C(14)-O(15)	1.368 (4)
C(5)-O(17)	1.373 (4)	O(15)-C(16)	1.357 (4)
C(7)-C(8)	1.468 (5)	O(17)-C(18)	1.417 (5)
C(7)-C(16)	1.340 (5)	O(19)-C(20)	1.427 (6)
C(8)-C(9)	1.460 (4)	O(22)-C(23)	1.433 (5)
C(8)-O(21)	1.229 (4)	O(24) -C(25)	1.424 (6)
C(9)-C(10)	1.393 (4)		

Table II

Bond Angles (°) of the Non-hyrogen Atoms of Isoflavone 2

C(6)-C(1)-C(7)	121.4 (3)	C(8)-C(9)-C(10)	122.3 (3)
C(2)-C(1)-C(7)	120.5 (3)	C(10)-C(9)-C(14)	117.2 (3)
C(2)-C(1)-C(6)	118.2 (3)	C(9)-C(10)-C(11)	121.9 (3)
C(1)-C(2)-C(3)	121.4 (3)	C(10)-C(11)-C(12)	119.4 (3)
C(2)-C(3)-C(4)	120.3 (3)	C(11)-C(12)-O(24)	124.8 (3)
C(3)-C(4)-O(19)	125.3 (3)	C(11)-C(12)-C(13)	120.5 (3)
C(3)-C(4)-C(5)	118.9 (3)	C(13)-C(12)-C(24)	114.7 (3)
C(5)-C(4)-O(19)	115.8 (3)	C(12)-C(13)-C(22)	121.4 (3)
C(4)-C-(5)-O(17)	115.3 (3)	C(12)-C(13)-C(14)	118.6 (3)
C(4)-C(5)-C(6)	120.9 (3)	C(14)-C(13)-O(22)	120.1 (3)
C(6)-C(5)-O(17)	123.8 (3)	C(9)-C(14)-C(13)	122.4 (3)
C(1)-C(6)-C(5)	120.3 (3)	C(13)-C(14)-O(15)	115.9 (3)
C(1)-C(7)-C(16)	118.5 (3)	C(9)-C(14)-O(15)	121.7 (3)
C(1)-C(7)-C(8)	122.6 (3)	C(14)-O(15)-C(16)	117.6 (3)
C(8)-C(7)-C(16)	118.9 (3)	C(7)-C(16)-O(15)	125.9 (3)
C(7)-C(8)-O(21)	123.5 (3)	C(5)-O(17)-C(18)	117.2 (3)
C(7)-C(8)-C(9)	114.8 (3)	C(4)-O(19)-C(20)	117.1 (3)
C(9)-C(8)-O(21)	121.8 (3)	C(13)-O(22)-C(23)	112.8 (3)
C(8)-C(9)-C(14)	120.4 (3)	C(12)-O(24)-C(25)	118.4 (3)

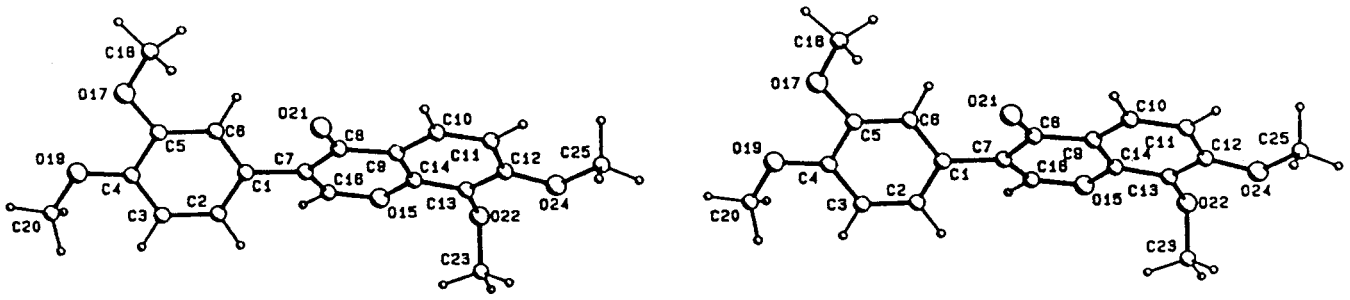


Figure II. Stereoscopic view of **2** with the number scheme used in crystallographic analysis.

The C(1)-C(7) bond linking the γ-pyrone and phenyl rings is 1.487(4) Å. This value could indicate that the resonance between these two rings causes this bond to have partial double bond character. This behaviour is similar to that observed in biphenyl bonds, with values of 1.4879(7) Å, quoted by Bastiansen and Traetteberger [14a] and 1.48 Å, suggested by Pauling [14b]. Rings A and B (see Scheme I) show significant deviations from planarity. The values of $\Omega^2[\Omega^2 = \Sigma(\Delta/\sigma)^2$, where Δ = atomic deviation from the calculated mean plane and σ = standard deviation of Δ] are 324.9 (six atoms) and 117.0 (six atoms), respectively. The dihedral angle between rings A and B is 5.0(1)°. The aryl group ring C attached at C(7) is less distorted from planarity [Ω^2 = 20.8 (six atoms)] and the dihedral angle between rings B and C is 32.0(1)°. This value compares well with those of 24.8°, 28°, and 32° reported for 5-hydroxy-7-methoxyflavone [15a], 3',5,5',6-tetramethoxyflavone [15b], and 3-benzoyl-5,8-dihydroxyflavone [15c], but differs significantly from values found in other flavone and isoflavone structures [13b], [13c], [16]. This can be easily rationalized by assuming that the twist angle about the bond connecting rings B and C increases due to the steric hindrance of the substituents attached at the *ortho* positions with respect to this bond.

As it can be seen from the torsion angles given in Table III, the methoxy groups at C(4), C(5), and C(12) are nearly coplanar with the benzene ring to which they are attached. Similar coplanarity of an aromatic ring with the methoxy groups adjacent to hydrogen atoms has been observed in mono- and *o*-dimethoxy aromatic compounds [17]. For the methoxy group at C(13) the torsion angles about C(13)-O(22) are about 90° (Table III) and show that the C(13)-O(22) bond is nearly perpendicular to ring A. Similar behaviour has been reported for an arrangement of three adjacent methoxy groups attached to the aromatic moiety [13a,18].

The significant biological activities exhibited by flavonoid structures led us to investigate the pharmacological properties of the isoflavone **2**. The acute toxicity of isoflavone **2** was evaluated using CFLP mice and Wistar rats. At a dose of 300 mg/Kg p.o. all the animals remained alive and without any symptomatology after a fourteen day period, indicating no acute toxicity of the compound at the tested dose. A pharmacological screening was carried out with this isoflavone in order to assess its pharmacological profile, according with the assays shown in Table IV.

Table III

Selected Torsion Angles (°) of Isoflavone 2

C(3)-C(4)-O(19)-C(20)	-5.1 (5)
C(11)-C(12)-O(24)-C(25)	-4.4 (5)
C(5)-C(4)-O(19)-C(20)	177.2 (5)
C(13)-C(12)-O(24)-C(25)	-175.8 (3)
C(4)-C(5)-O(17)-C(18)	171.0 (3)
C(12)-C(13)-O(22)-C(23)	-91.1 (4)
C(6)-C(5)-O(17)-C(18)	-9.2 (5)
C(14)-C(13)-O(22)-C(23)	-88.4 (4)

Isoflavone **2** showed significant pharmacological activity as antiinflammatory in the carrageenan oedema test, while was inactive in other tests carried out. The antiinflammatory activity was further investigated in order to confirm the results and to establishe a dose-effect relationship. The carrageenan-induced rat paw oedema was the experimental model used [28]. Results are shown in Table

Table IV

Pharmacological Screening of the Isoflavone 2

Activity	Experimental model	Animal	Dose (mg/Kg)	Reference
Anticonvulsant	Anti-metrazol	mouse	300	19
Antithypoxic	Hypobaric hypoxia	mouse	300	20
Anxiolytic	Betzold-Jarisch	mouse	100	21
Analgesic	Writhing test	mouse	300	22
Hypolipaemic	Triton-induced Hyperlipidaemia	mouse	300	23
Antihistaminic	Capillary permeability	rat	50	24,25
Antiallergic	PCA	rat	50	24,26
Antiemetic	Apomorphine stereotype	rat	50	27
Antiimflammatory	Carrageenan oedema	rat	300	28
Antisecretory	Piloric ligation	rat	100	29

V. Statistically significant activity was found for doses of 200 and 300 mg/Kg in relation to the control group with a dose-effect relationship.

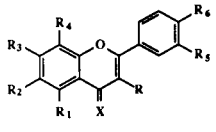
Table V
Antiinflammatory Activity of the Isoflavone 2

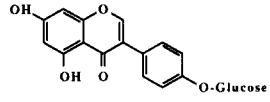
Treatment(mg/Kg)	Paw volume increase (mean SE, %)	Inhibition (%)
Control	72.65±7.65	-
100	64.56±1.86	11.14
200	51.99±2.01	28.44 [a]
300	42.91±9.42	40.94 [a]

[a] $p<0.05$ (student's t-test).

In recent years, flavonoids have been reported to posses antiinflammatory properties against carrageenan induced paw oedema in rats and increase of permeability [30]. Compounds in Figure III have been tested in rats and exhibited a 25-64% inhibition of the phlogistic response in doses below 100 mg/Kg. Sophoricoside, first isoflavone tested against carrageenic oedema, exerted high efficiency in dose of 25 mg/Kg. The results related to the isoflavone **2** are worthy of interest as, to our knowledge, this is one of the few examples of isoflavones efficient as antiinflammatory, and the only one exhibiting complete alkylation of hydroxyl groups.

Figure III





Sophoricoside

Gossipyne X=O, R=R1=R3=R5=R6=OH,R2=H,R4=O-Glucose
Nepitrin X=O, R=R3=R4=H, R1=R5=R6=OH,R2=OMe
Hypoaletin X=O, R=R2=H,R1=R3=R5=R6=OH,R2=H,R4=O-Glucose
Apigenin X=O, R=R2=R4=R5=H,R1=R3=OMe,R6=OH
Fisetin X=O, R=R3=R5=R6=OH,R1=R2=R4=H
Taxifoline X=O, R=R2=R3=R5=R6=OH,R1=R4=H
(+)Catechin X=H2,R=R1=R3=R5=R6=OH,R2=R4=H

EXPERIMENTAL

Chemistry.

Melting points were determined on a Büchi apparatus and are uncorrected. The ir spectra were measured in potassium bromide on a Perkin-Elmer 1430 spectrophotometer and only noteworthy absorptions (cm^{-1}) are reported. The pmr and cmr spectra were recorded at room temperature on a Bruker AC-250 spectrometer operating at 250.13 MHz for proton and at 62.83 MHz for carbon. Chemical shifts given in δ values were measured respect to tetramethylsilane. Tlc was performed on silica gel 60 plates (0.2 mm layer, GF-254, Merck), using dichloromethane-ethyl acetate 95:5 as eluent. Visualization was accomplished by uv light or by spraying with Dragendorff's reagent [31]. Chemicals and solvents were purified according to method described by Perrin and col. [32].

7,8,3',4'-Tetramethoxyisoflavone (2).

Method A.

A solution of **1** (4 g, 12 mmoles) in anhydrous ethyl formate (100 ml) was added dropwise to powdered sodium (4 g, 17.4 mmoles) at 0° and then the mixture was left at 0° for 12 hours. Evaporation of the excess of ethyl formate afforded a semi-solid crude which was crystallized from a 1:1 mixture of ethyl acetate-light petroleum ether, thus affording the intermediate 2-hydroxy-7,8,3',4'-tetramethoxyisoflavanone **3** (2.16 g, 50%), as a white solid, R_F = 0.3, mp 180-181°; ir (potassium bromide): ν max 3480 (O-H), 1675 (C=O) cm^{-1}. The pmr and cmr spectra of **3** in deuteriochloroform showed that a mixture of two diastereomeric cyclic forms **3b** and **3a** is present; pmr: δ 7.76-7.69 (1H, m, H-5), 6.89-6.77 (3H, m, H-2', H-5', and H-6'), 6.72-6.67 (1H, m, H-6), 5.95* and 5.91† (1H, 2d, J = 3.4 Hz, and J = 2.9 Hz, H-2), 4.12† and 3.91* (1H, 2d, J = 2.9 Hz, and J = 3.4 Hz, H-3), 3.95†, 3.94†, 3.90*, 3.89*, 3.87†, 3.83*, and 3.82† (12H, 8s, 4 x MeO), 1.25 (1H, br s, OH, disappears with deuterium oxide) [* corresponds to major isomer **3b** and † to minor isomer **3a**]; cmr: δ 190.26, 190.08 (C=O), 159.22, 159.03 (C_7), 151.71, 151.11 (C_9), 149.12, 148.93, 148.91, 148.73 ($C_{3'}$ and/or $C_{4'}$), 137.15, 137.07 (C_8), 126.71, 124.72 ($C_{1'}$), 123.56, 123.18, 122.70, 120.32 (C_5 and/or $C_{6'}$), 116.13, 115.35 (C_{10}), 113.59, 111.69, 111.44, 111.19 ($C_{2'}$ and/or $C_{5'}$), 106.16, 106.04 (C_6), 98.67, 98.11 (C_2), 57.82, 57.08 (C_3), 61.23, 56.47, 56.24, 55.96, 55.86 (CH_3O). The intermediate **3** (2.16 g, 6 mmoles) was refluxed with acetic anhydride (70 ml) for 30 minutes. The reaction mixture was poured into water and extracted with dichloromethane (3 x 50 ml). The dried organic extracts (sodium sulfate) were evaporated to dryness to obtain quantitatively the isoflavone **2**, which was recrystallized from ethanol, R_F = 0.4, mp 168-170° [lit [6] 168-170° (ethanol)].

Method B.

To a solution of the ketone **1** (2 g, 6 mmoles) in anhydrous benzene (25 ml), *N,N*-dimethylformamide dimethylacetal (1 ml, 6.6 mmoles) was added dropwise at room temperature. The reaction mixture was refluxed for 6 hours until total consumption of the starting material (tlc monitoring). The mixture was put aside at 0° for 12 hours and the crystals that separated were filtered and recyrstallized from ethanol to give the isoflavone **2** (1.65 g, 80%).

X-Ray Diffraction Study of 7,8,3',4'-tetramethoxyisoflavone (2).

The isoflavone **2** was crystallized from ethanol as colourless plate-like crystals and their crystal data are as follows: $C_{19}H_{18}O_6$, Mr = 342.4, monoclinic, $P2_1/n$, a = 28.051(9), b = 6.750(1), c = 8.833(2)Å, β = 94.71(2)°, V = 1667(1)$Å^3$, Z = 4, D_x = 1.36$Mg.m^{-3}$, CuKα radiation, λ = 1.5418Å, μ = 0.86 mm^{-1}, F(000) = 720, room temperature, R = 0.044 for 1519 observed reflections. Data were collected from a crystal of approximately 0.1 x 0.1 x 0.05 mm. Cell parameters were determined using a

least squares procedure involving the angles setting of 25 reflections ($20° \leq 2\theta \leq 40°$). The intensities of 2179 independent reflections were measured on a Nonius CAD-4 diffractometer, using graphite monochromated CuKα radiation, ω-2θ scans ($\Delta 2\theta = 2.5 + 0.5\ \mathrm{tg}\theta$) up to $2\theta = 56°$. One standard reflection (060) measured every 50 reflections showed only a random deviation from its mean intensity. Lorentz and polarization but not absorption corrections were applied. A total of 1519 reflections ($I \geq 2.4\,\sigma(I)$) were considered observed and were included in the refinement. The index range was h -29 → 29, k 0 → 7, l 0 → 9. The structure was solved with MULTAN 11/84 [33] and the least squares refinement was carried out using SHELX76 [34] and completed with anisotropic thermal parameters for non-H atoms. H-atoms at idealized positions were included in the calculation and were assigned isotropic thermal parameters equal to the overall isotropic thermal parameters of the structure. Non of the H-atom parameters were refined. The final R values were R = 0.044 and R_w = 0.055. Scattering factors from SHELX76 [34] were used. The maximum Δ/σ for non-H atoms was 0.97. Maximum and minimum electron densities in the final difference map were +0.14 and −0.22 e Å^{-3}.

Pharmacological Activity of 7,8,3',4'-Tetramethoxyisoflavone (**2**).

Preliminary screenings were done using CFLP mice and Wistar rats according to the protocol described in the previous section (see Table IV). Antiinflammatory activity was tested following the carrageenan-induced rat paw oedema experimental model [20a]. Plantar oedema was induced in Wistar rats by injection of 0.1 ml of 1.5% carrageenan in saline. Test compound suspended in 1% CMC aqueous solution was administered orally at doses of 100, 200, and 300 mg/Kg of body weight, one hour before the carrageenan injection. Six animals were used in each experiment group. The volume of oedema was detemined three hours after carrageenan challenge (see Table V).

Supplementary Material Available.

Lists of structure factors, fractional atomic coordinates and anisotropic thermal parameters for the non-hydrogen atoms, hydrogen atoms coordinates, bond lengths and bond angles, least-squares planes, and torsion angles are available directly from the author.

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