FUSED HETEROCYCLIC SISTEMS. NOTE I. PYRANO[3,4-c]PYRROLE-4,7(2H,6H)-DIONE. SYNTHESIS AND GENERAL PHARMACOLOGICAL SCREENING

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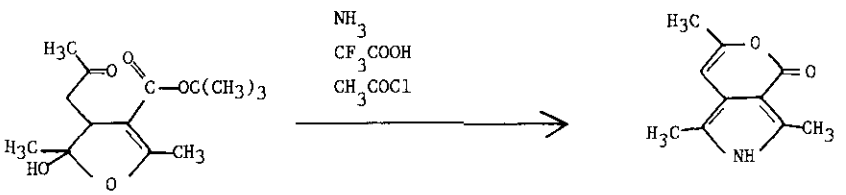
Abstract - A general synthesis of pyrano[3,4-c]pyrrole-4,7(2H,6H)-dione derivatives is described. The structures of epoxypropyl intermediates and of final compounds are demonstrated by ^{13}C-nmr analysis. There are also reported the results of a general pharmacological screening on the compounds of the title.

It is reported to our knowledge only one synthesis of pyrano[3,4-c]pyrrol-4(2H)-one. In fact Giardina et al.[1] have obtained the 1,3,6-trimethylpyrano[3,4-c]pyrrol-4(2H)-one (II) by action of aqueous ammonia on the t-butyl ester of structure (I) and by subsequent hydrolysis to the correspondent carboxylic acid.



(I) (II)

We report here an original general synthesis of this heterocyclic system.

We applied the Darzens reaction to the ethyl 4-chloroacetyl-2,5-dimethyl-1H-pyrrole-3-carboxylate (IV) obtained by action of chloroacetyl chloride on the ethyl 2,5-dimethyl-1H-pyrrole-3-carboxylate (III) in presence of $AlCl_3$. The compound (IV) was condensed with the para-substituted aromatic aldehydes (Va-g) to obtain the epoxypropyl derivatives (VIa-g). The subsequent action of ethanol saturated with hydrogen chloride caused compounds cyclization giving (VIIIa-g). (Scheme 1).

The structures of the derivatives (VIa-g) and (VIIIa-g) were confirmed by elemental analysis and spectroscopic data (ir, nmr, mass).

The epoxide structure of compounds (VIa-g) was demonstrated by nmr analysis. Besides the signals of the carbethoxy group, pyrrole methyl groups, and pyrrole NH proton, the ^{1}H-nmr spectra performed at 60 MHz in deuteriated dimethyl sulfoxide solutions showed all derivatives a singlet centered at δ 3.9 ~ 4.0 integrated for two protons, together with the resonances of the variable R substituent. (Table)

The nmr spectra at higher frequency allowed in some cases to separate in the two superimposed epoxide protons. Particularly, the ^{1}H-nmr spectrum at 90 MHz of trifluoromethyl derivative (VIe) showed two distinct doublets at δ 4.04 and 4.15 with 2.0 Hz of vicinal coupling constant. Also the ^{1}H-nmr spectrum of the methoxy derivative (VIg) performed at 300 MHz gave, regarding to the epoxide protons, two doublets at δ 3.86 and 3.92 with J = 1.8 Hz, each of the signals corresponding to one proton. The small value of this coupling constant is consistent with a trans-isomer, as reported in literature data[2,3]. In accordance with this structure, the ^{13}C-nmr spectra of the same solutions exhibited the epoxide carbon atoms two absorptions at ca. 58 and ca. 62 ppm, doublets in off-resonance experiments, together with the signals of the pyrrole ring, methyl groups at pyrrole Cα, carbethoxy and carbonyl groups at pyrrole Cβ, and of the variable R substituent. When necessary, the assignment of ^{13}C-nmr signals was made by a proton fully coupled spectra; particularly the assignment of the epoxide carbon atoms come from the higher multiplicity of the signal at higher field, which was assigned to C-1 carbon atom of the epoxypropyl ring.

The proton fully coupled ^{13}C-nmr technique was conclusive for the assignment of structure of the pyranopyrroledione derivatives. In fact, the off-resonance spectra of the compounds (VIII) showed the singlets for quaternary carbon atoms without one-bond interactions of the condensed structure, the quartets for the pyrrole methyl groups and a doublet at ca. 115 ppm together with

the signals of the variable R substituent. These data do not allow to discriminate structures with exocyclic CH from those with endocyclic CH, but the proton totally coupled ^{13}C-nmr spectra revealed for the signal at *ca.* 115 ppm a multiplicity of doublets (^{1}J 157 Hz) and of triplets (^{3}J 4.5 Hz), have indicated the presence of benzylidene group at C-6; and the multiplicity (double triplet with one one-bond interaction and two three-bond interactions) of the C-2',6' carbon atoms resonance revealed the presence of the benzylidene CH.

It must be noticed that compounds (VIII) were very scarcely soluble and only for (VIIIa,e,f,g) derivatives it was possible to prepare deuteriated pyridine solutions. Furthermore due to this fact some resonances were overlapped by solvent signals and only for the derivative (VIIIg), the concentration of the solution was sufficient to realize a proton fully coupled ^{13}C-nmr spectrum.

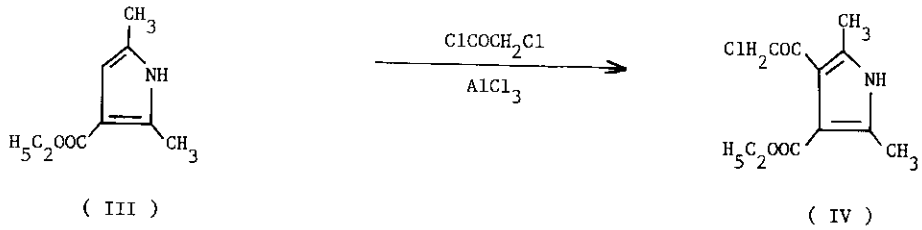
We suggest that the pyrano[3,4-c]pyrrole-4,7-dione could be obtained through the formation of the intermediate (VIIa-g), which is not isolable in our case. Our hypothesis is supported by Kohler and Barnes[4], who demonstrated that in the case of benzalacetophenone oxide by reaction with mineral acids obtained the enolic form of the α diketone.

By treatment of compound (VIIIa) with KOH we obtained the acid (IX), that could have the structure shown in the scheme 2 on the basis of experimental results (ir, mass, elemental analysis). We can not characterize the structure by nmr analysis because the compound is not stable in all the solvents we used, due probably to deuteritation.

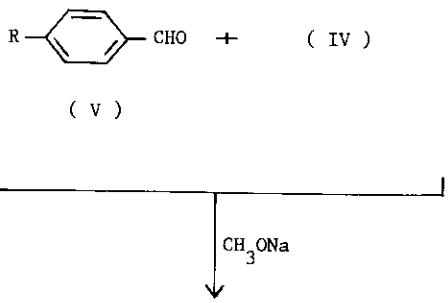
Finally the presence of the double bond on (VIIIa-g) was confirmed by the fact that (VIIIf) easily absorbs one equivalent mole of hydrogen obtaining (X). (Scheme 2)

The structural relationship of the compounds (VIII) to the benzoisocoumarines, of which biological properties are well known, prompted us to a general pharmacological screening.

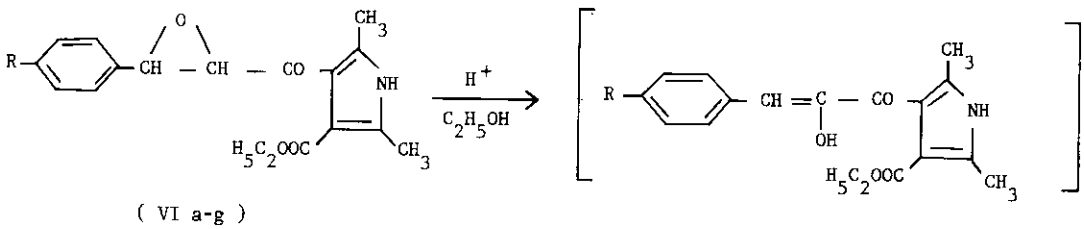
Our results showed that the derivatives (VIII) did not induce remarkable behaviour modification according to Irwin's assay at doses of 50 and 100 mg/kg i.p. A moderate sedation, motor incoordination and ataxia were observed for a period of 6-12 h after injection of 250 mg/kg and over for all the compounds tested. Death generally occurred at 2-4 days postdrug (LD_{50} = about 400-500 mg/kg i.p.). None of the compounds exhibited any analgesic and antiinflammatory action at doses of 10-50 and 100 mg/kg i.p. As far as relatively high doses (50-100 mg/kg i.p.), the test compounds showed no effect on the central nervous system in mice. All different tests carried were negative.



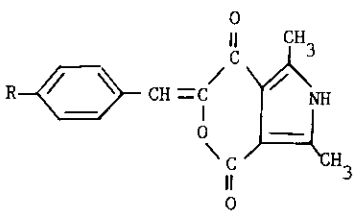
(III) (IV)



a: R = H
b: R = Br
c: R = Cl
d: R = F
e: R = CF_3
f: R = CH_3
g: R = OCH_3

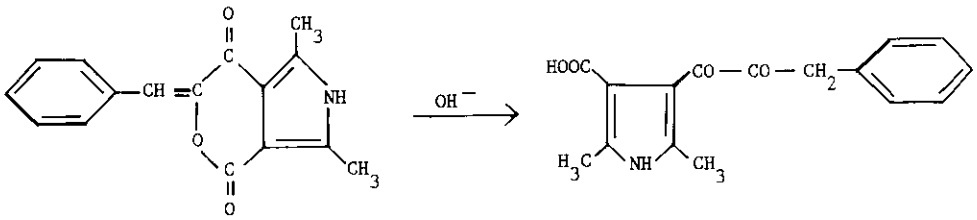


(VI a-g) (VII a-g)

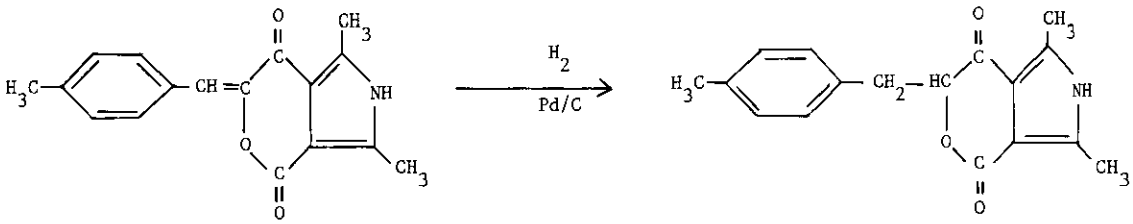


(VIII a-g)

SCHEME 1



(VIII a) (IX)



(VIII f) (X)

SCHEME 2

Table: ^{13}C- and ^{1}H- (in parentheses) nmr parameters[a] of compounds VIa-g in DMSO-d_6 solutions

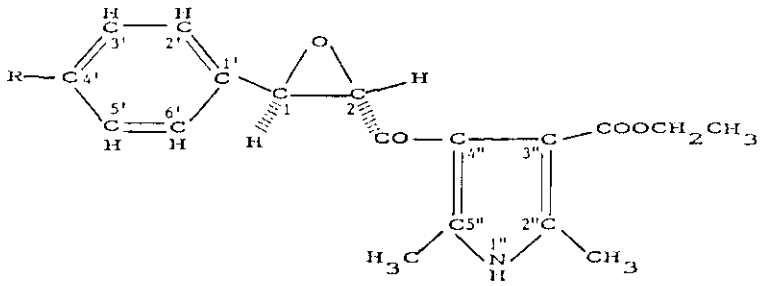
	C-1(d)[b]	C-2(d)	C-1'(s)	C-2',6'(d)	C-3',5'(d)	C-4'	R	CO(s)	C-2"(s)
VIa (R=H)	58.21	62.50	136.25	126.09	128.41	128.54		192.82	134.22♦
	(3.96,2H,s)			(7.27,5H,s)					
VIb (R=Br)	57.66	62.39	135.85	128.34	131.39	121.74		192.54	134.28♦
	(4.0,2H,s)			(7.27,2H,d) (J 8.5 Hz)	(7.60,2H) (d J 8.5 Hz)				
VIc (R=Cl)	57.58	62.43	135.40°	128.07●	128.50●	133.22°		192.58	134.29♦
	(3.98,2H,s)			(7.23,4H,s)					
VId[e] (R=F)	57.87	62.59	132.63	128.29	115.35	162.41		192.76	134.42♦
	(3.95,2H,s)			(6.90-7.50,4H,m)					
VIe[f] (R=CF_3)	57.63	62.73	141.26	126.88	125.23	129.23	124.20	192.29	134.42♦
	(4.04,1H,d,J≅2Hz) (4.15,1H,d,J≅2Hz)			(7.68,2H,d,J 8.5 Hz) (7.80,2H,d,J 8.5 Hz)					
VIf (R=CH_3)	58.40	62.57	133.34	126.08	129.05	138.01	20.75 q (2.26,3H,s)	193.08	134.36♦
	(3.92,2H,s)			(7.15,4H,s)					
VIg (R=OCH_3)	58.37	62.54	128.13	127.59	113.97	159.75	55.11 q (3.74,3H,s)	193.24	134.38♦
	(3.86,1H,d,J 1.8Hz) (3.92,1H,d,J 1.8Hz)			(7.24,2H,d) (J 8.7 Hz)	(6.91,2H) (d J 8.7 Hz)				

[a] Chemical shifts values are given in ppm from $(CH_3)_4Si$. Values with the same symbol may be interchanged in each compound. All the ^{13}C-nmr spectra were recorded at 50 MHz. ^{1}H-Nmr spectra were recorded at 60 MHz in the case of VIa, c, d, f, at 90 MHz for VIb, e, and at 300 MHz for VIg.

[b] The multiplicity is derived from off-resonance experiments; for C-4' the multiplicity was singlet or doublet depending on the R subsituent.

[c] Proton signal exchangeable with deuterium oxide.

[d] Signal overlapped by the resonance of the epoxide protons.



C-3"(s)	C-4"(s)	C-5"(s)	COO(s)	OCH_2(t)	$OCH_2\underline{CH_3}$(q)	C-2"-$\underline{CH_3}$(q)	C-5"-$\underline{CH_3}$(q)	NH[c]
110.39	119.41	132.33♦	164.31	59.35 (~4.0)[d]	14.06 (1.15,3H) (t J 7.0 Hz)	12.29* (2.32□,3H,s)	11.75* (2.20□,3H,s)	(11.70,1H) (s broad)
110.43	119.45	132.54♦	164.33	59.44 (~4.0)[d]	14.12 (1.12,3H) (t J 7.2 Hz)	12.40* (2.30□,3H,s)	11.84* (2.20□,3H,s)	(11.65,1H) (s broad)
110.44	119.43	132.53♦	164.32	59.44 (~4.0)[d]	14.12 (1.15,3H) (t 7.0 Hz)	12.38* (2.32□,3H,s)	11.83* (2.20□,3H,s)	(11.60,1H) (s broad)
110.53	119.61	132.63♦	164.46	59.47 (~4.0)[d]	14.09 (1.12,3H) (t J 7.0 Hz)	12.37* (2.32□,3H,s)	11.85* (2.18□,3H,s)	(11.70,1H) (s broad)
110.53	119.60	132.88♦	164.41	59.44 (~4.0)[d]	14.00 (1.12,3H) (t J 7.2 Hz)	12.36* (2.35□,3H,s)	11.84* (2.24□,3H,s)	(11.60,1H) (s broad)
110.54	119.57	132.40♦	164.44	59.44 (~4.0)[d]	14.12 (1.15,3H) (t J 7.0 Hz)	12.38* (2.32□,3H,s)	11.82* (2.20□,3H,s)	(11.65,1H) (s broad)
110.56	119.62	132.41♦	164.47	59.46 (4.10,1H,m) (3.96,1H,m)	14.13 (1.14,3H) (t J 7.2 Hz)	12.37* (2.32□,3H,s)	11.83* (2.19□,3H,s)	(11.60,1H) (s broad)

[e] The proton decoupled ^{13}C-nmr spectrum exhibits the resonances of C-4' carbon atom as doublet with $^1J_{C,F}$ 245.7 Hz, C-3',5' as doublet with $^2J_{C,F}$ 22.1 Hz, and C-2',6' as doublet with $^3J_{C,F}$ 6.7 Hz.

[f] The proton decoupled ^{13}C-nmr spectrum exhibits the resonances of CF_3 carbon atom as quartet with $^1J_{C,F}$ 271.8 Hz, C-4' as quartet with $^2J_{C,F}$ 31.8 Hz, and the C-2',6' absorption broadened from C-F three-bond interactions.

EXPERIMENTAL

CHEMISTRY

All melting points were taken on a Büchi-Tottoli micro melting point apparatus and are uncorrected. Ir spectra were recorded in nujol mull with a Perkin Elmer Infrared 137 E spectrophotometer. ^{1}H-Nmr spectra were recorded on EM-360A or EM-390 or XL-300 Varian spectrometers in DMSO-d_6 solutions for compounds (VI) and on a Bruker WP-80 instrument in pyridine-d_5 solutions for compounds (VIII), using TMS as internal standard. ^{13}C-Nmr spectra were recorded on a Bruker-200 or Varian FT-80A spectrometers, respectively operating at 50 MHz and 20 MHz. The ^{13}C-nmr chemical shifts values were taken from fully decoupled spectra; off-resonance and proton coupled experiments were also performed in the structures determination. Mass spectra were recorded on a JEOL-JMS-01-SG-2 spectrometer operating with an ionizing electrons beam at 75 eV. Elemental analyses for C, H, N, Cl, Br, F were within 0.16% of the calculated values and were determined by the Institut de Chimie Pharmaceutique-Service de microchimie-Université de Geneve.

General procedure for the synthesis of compounds (VIa-g)

25 ml of dry methanol in which there was dissolved 50 mmol of Na have been added, drop by drop under magnetical stirring at 20°C, to the solution of (IV) (22 mmol) in 80 ml of dry methanol and (24 mmol) of the appropriate aldehyde. After standing overnight the solution was brought to a small volume under reduced pressure. The compound (VI) was precipitated with water and separated by filtration and recrystallized from aqueous ethanol.

Ethyl 2,5-dimethyl-4-(1-oxo-3-phenyl-2,3-epoxypropyl)-1H-pyrrole-3-carboxylate (VIa):

mp 150°C; yield 60%; ir (cm^{-1}): 3260, 1695, 1645; ms: m/z 313 (M^+)

Ethyl 2,5-dimethyl-4-[3-(4-bromophenyl)-1-oxo-2,3-epoxypropyl]-1H-pyrrole-3-carboxylate (VIb):

mp 160°C; yield 78%; ir (cm^{-1}): 3260, 1690, 1630, 1510; ms: m/z 392 (M^+)

Ethyl 2,5-dimethyl-4-[3-(4-chlorophenyl)-1-oxo-2,3-epoxypropyl]-1H-pyrrole-3-carboxylate (VIc):

mp 152°C; yield 58%; ir (cm^{-1}): 3260, 1690, 1630; ms: m/z 347 (M^+)

Ethyl 2,5-dimethyl-4-[3-(4-fluorophenyl)-1-oxo-2,3-epoxypropyl]-1H-pyrrole-3-carboxylate (VId):

mp 141°C; yield 88%; ir (cm^{-1}): 3260, 1700, 1645, 1510; ms: m/z 331 (M^+)

Ethyl 2,5-dimethyl-4-[1-oxo-3-(4-trifluoromethylphenyl)-2,3-epoxypropyl]-1H-pyrrole-3-carboxylate (VIe):

mp 195°C; yield 87%; ir (cm^{-1}): 3280, 1705, 1645, 1590; ms: m/z 381 (M^+).

Ethyl 2,5-dimethyl-4-[3-(4-methylphenyl)-1-oxo-2,3-epoxypropyl]-1H-pyrrole-3-carboxylate (VIf):

mp 155°C; yield 87%; ir (cm^{-1}): 3240, 1700, 1660; ms: m/z 327 (M^+)

Ethyl 2,5-dimethyl-4-[3-(4-methoxyphenyl)-1-oxo-2,3-epoxypropyl]-1H-pyrrole-3-carboxylate (VIg):

mp 157°C; yield 81%; ir (cm^{-1}): 3220, 1700, 1615, 1510; ms: m/z 343 (M^+)

General procedure for the synthesis of compounds (VIIIa-g)

20 ml of dry ethanol saturated with hydrogen chloride were added to a suspension of 20 mmol of (VI) in dry ethanol (ca. 30 ml). The suspension was left under magnetical stirring at room temperature. The progress of the reaction was monitored by TLC (petroleum ether 40°-60° - ethyl acetate 1:1), and, when the starting material disappeared, the mixture was neutralized with sat. sodium bicarbonate and the precipitate was filtered. The resulting residue was purified by recrystallization from methanol.

1,3-Dimethyl-6,6-(phenylmethylene)pyrano[3,4-c]pyrrole-4,7(2H,6H)-dione (VIIIa):

mp > 300°C; yield 80%; ir (cm^{-1}): 3250, 1745, 1380; ms: m/z 267 (M^+); ^{1}H-nmr (C_5D_5N) [80 MHz] δ: 2.63(3H,s,CH_3), 2.67(3H,s,CH_3), 7.35(1H,s,Olefinic-H), 7.30-7.40(3H,m,Ph-H), 8.00-8.20(2H,m, Ph-H), 13.5(1H,s broad,NH). ^{13}C-Nmr (C_5D_5N) [50 MHz] ppm: 11.85 and 12.40 (2 CH_3), 107.86 and 116.28 (2 Pyrrole Cβ), 115.38(Olefinic CH), 129.07, 129.45, and 131.72 (Ph CH), 133.76, 148.98, 157.85, and 175.49 (detected signals for quaternary carbons, the others being overlapped by solvent signals).

1,3-Dimethyl-6,6-[(4-bromophenyl)methylene]pyrano[3,4-c]pyrrole-4,7(2H,6H)-dione (VIIIb):

mp > 300°C; yield 85%; ir (cm^{-1}): 3250, 1715, 1460, 1380; ms: m/z 346 (M^+).

1,3-Dimethyl-6,6-[(4-chlorophenyl)methylene]pyrano[3,4-c]pyrrole-4,7(2H,6H)-dione (VIIIc):

mp > 300°C; yield 60%; ir (cm^{-1}): 3240, 1720, 1460, 1380; ms: m/z 301 (M^+).

1,3-Dimethyl-6,6-[(4-fluorophenyl)methylene]pyrano[3,4-c]pyrrole-4,7(2H,6H)-dione (VIIId):

mp > 300°C; yield 88%; ir (cm^{-1}): 3240, 1720, 1460, 1380; ms: m/z 285 (M^+).

1,3-Dimethyl-6,6-[(4-trifluoromethylphenyl)methylene]pyrano[3,4-c]pyrrole-4,7(2H,6H)-dione (VIIIe):

mp > 300°C; yield 90%; ir (cm^{-1}): 3260, 1730, 1460, 1380; ms: m/z 335 (M^+); ^{1}H-nmr (C_5D_5N)

[80 MHz] δ: 2.64(3H,s,CH_3), 2.68(3H,s,CH_3), 7.28(1H,s,Olefinic-H), 7.61(2H,d,Jo = 8.2 Hz, Ph-H), 8.15(2H,d,Jo = 8.2 Hz, Ph-H), 13.5(1H,s broad,NH). ^{13}C-Nmr (C_5D_5N) [50 MHz] ppm: 11.84 and 12.36 (2 CH_3), 107.41 and 116.22(2 Pyrrole Cβ), 112.90(Olefinic CH), 125.68 and 131.72(Ph CH), 136.89, 137.54, 157.27, and 174.95 (detected signals for quaternary carbons, the others being overlapped by solvent signals).

1,3-Dimethyl-6,6-[(4-methylphenyl)methylene]pyrano[3,4-c]pyrrole-4,7(2H,6H)-dione (VIIIf):

mp > 300°C; yield 87%; ir (cm^{-1}): 3220, 1720, 1460, 1380; ms: m/z 281 (M^+). ^{1}H-Nmr (C_5D_5N) [80 MHz] δ: 2.20(3H,s,Ph CH_3), 2.64(3H,s,Pyrrole CH_3), 2.68(3H,s,Pyrrole CH_3), 7.15(2H,d,Jo = 8.1 Hz, Ph-H), 7.35(1H,s,Olefinic-H), 8.05(2H,d,Jo = 8.1 Hz,Ph-H), 13.5(1H,s broad,NH). ^{13}C-Nmr (C_5D_5N) [50 MHz] ppm: 11.79 and 12.30(2 Pyrrole CH_3), 21.24(Ph CH_3), 108.05 and 116.41(2 Pyrrole Cβ), 115.39 (Olefinic CH), 129.75 and 131.72(Ph CH), 139.51, 148.78, 157.71, and 175.39 (detected signals for quaternary carbons, the others being overlapped by solvent signals).

1,3-Dimethyl-6,6-[(4-methoxyphenyl)methylene]pyrano[3,4-c]pyrrole-4,7(2H,6H)-dione (VIIIg):

mp > 300°C; yield 80%; ir (cm^{-1}): 3220, 1740, 1460, 1380; ms: m/z 297 (M^+); ^{1}H-nmr (C_5D_5N) [80 MHz] δ: 2.63 (3H,s,CH_3), 2.68(3H,s,CH_3), 3.66(3H,s,OCH_3), 6.93(2H,d,Jo = 8.8 Hz, Ph-H), 7.34(1H, s,Olefinic-H), 8.11(2H,d,Jo = 8.8 Hz,Ph-H), 13.50(1H,s broad,NH); ^{13}C-nmr (C_5D_5N) [20 MHz] ppm: 11.86(q,^{1}J=129.3 Hz,CH_3), 12.36(q,^{1}J=129.3 Hz,CH_3), 55.30(q,^{1}J=144.3 Hz,OCH_3), 108.29(m without one-bond interactions,Pyrrole Cβ), 114.72(dd,^{1}J=159.5 and ^{3}J=4.5 Hz, C-3' and C-5'), 115.45(dt, ^{1}J=156.3 and ^{3}J=4.5 Hz, Olefinic CH), 116.70(m without one-bond interactions, Pyrrole Cβ), 133.52 (dt,^{1}J=160.1 and ^{3}J=6.6 Hz, C-2' and C-6'), 126.63, 136.17, 148.14, 157.91, 160.87, and 175.51 (resonances without one-bond interactions, the others being overlapped by solvent signals).

Hydrolysis of (VIIIa): 2,5-dimethyl-4-(1,2-dioxo-3phenylpropyl)-1H-pyrrole-3-carboxylic acid (IX).

A mixture of (VIIIa) (1g, 0,0037 mol), ethanol (2 ml) and 3% aqueous potassium hydroxide (5 ml) was stirred for 24 h at room temperature. After pouring on the crushed ice, the solution was made acidic with hydrochloric acid and the resulting yellow precipitate was separated by filtration and recrystallized from ethyl acetate; mp 201°C; yield 87%; ir (cm^{-1}): 3400, 3270, 1665(COOH); ms: m/z 285 (M^+).

Hydrogenation of (VIIIf): 1,3-dimethyl-6-phenylmethylpyrano[3,4-c]pyrrole-4,7(2H,6H)-dione (X).

A mixture of (VIIIf) (0.6 g, 0.0021 mol), ethanol (200 ml), and 2g of 10% Pd/C was hydrogenated at room temperature. After 1.5 h, the catalyst was removed and evaporation of ethanol under reduced

pressure left white needles; mp 232°C from ethanol; yield 72%; ir (cm^{-1}): 3200, 1715, 1465; ms: m/z 283 (M^+); ^{1}H-nmr (DMSO-d_6) δ: 2.20(3H,s,CH_3), 2.32(3H,s,Pyrrole CH_3), 2.40(3H,s,Pyrrole CH_3), 3.10(2H,d,J=5.0 Hz,CH_2), 5.15(1H,t,J=5.0 Hz, CH), 7.00(4H,s, Ar-H), 12.30(1H,s broad, NH).

Pharmacology

The compounds described in this paper were screened for their pharmacological activities and for general behavioural effects. Swiss male mice (25-30 g) were used. All compounds were suspended in 0.5% aqueous methyl cellulose solution and administered intraperitoneally at 10 ml/kg. The animals were starved for about 15 h before administration.

1. Behavioural effects and acute toxicity in mice. The Irwin's multidimensional screening-evaluation procedure[5] was used on groups of five animals. The compounds were administered i.p. at four levels of doses (50-100-250-500 mg/kg). The approximate LD_{50} was obtained from mortality 7 days later.

2. Analgesic activity. a) Hot plate test[6]: mice were placed individually on a copper plate, kept at 55°C. The compounds were administered to groups of five animals. Measurements were carried out 0.5, 1.0, 1.5, and 2 h after treatment, comparing them with a control group. b) Phenylquinone writhing test[7]: 60 min after the oral administration of test drugs, groups of six mice were injected i.p. with 0.25 ml/mouse with a 0.02% aqueous ethanol solution of phenylquinone. The analgesic effect was expressed as percentage of protection in comparison with the control group.

3. Antiinflammatory activity. Antiinflammatory activity was assessed by means of the carrageenin induced oedema test by method of Sandrini and Zanoli[8].

Effects on the central nervous system. The central effects of test compunds were investigated in mice (4 animals/group) using differents standard tests. Drugs were administered i.p. at 50 and 100 mg/kg. The following tests were carried out 30 min after the treatment. a) Anticholinergic activity was evaluated as described by Leszkovszky and Tardos[9]. b) Duration of barbiturate sleep: pretreated mice were injected i.p. with barbital (160 mg/kg). c) Anticonvulsivant activity: mice were given s.c. with an aqueous solution of pentylenetetrazole (100 mg/kg). The antagonism of pentylenetetrazole-induced clonic convulsions was evaluated for a period of 20 min. d) Antagonism of reserpine-induced ptosis: mice received i.p. reserpine (2.5 mg/kg). Ptosis

scores were evaluated as described by Rubin et al.[10]. e) Catalepsy: pretreated mice were placed so that their fore-paws rested on a 5-cm high pedestal and it was recorded the number of seconds to a maximum of 30 sec in which the animal remained in this position.

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