The combined filtrates from the isolation and recrystallization of the above (+)-**3a** di-*p*-toluoyl-D-tartrate were evaporated to dryness in vacuo. The residue was dissolved in 30 mL of H_2O and made alkaline with NH_4OH. Extraction with Et_2O and evaporation of the dried extracts left 0.86 g (3.5 mmol) of base, principally (−)-**3a**, which was warmed to solution with 20 mL of Me_2CO and 5 mL of MeOH containing 1.42 g (3.5 mmol) of di-*p*-toluoyl-L-tartaric acid hydrate. The filtered solution, combined with 5 mL of Me_2CO "washing", was concentrated to 5 mL and cooled overnight at 0 °C, giving 1.8 g of salt. It was recrystallized twice from MeOH, yielding 1.65 g (75%), mp 151–154 °C dec. This was converted to (−)-**3a** (H_2O–NH_4OH–Et_2O) as described in the isolation of (+)-**3a**, giving, after recrystallization from Me_2CO, 0.6 g: mp 175–176 °C; $[\alpha]^{25}_D$ −49.0° (*c* 1.05, EtOH). Anal. ($C_{16}H_{23}NO$) C, H, N.

Methiodide (7) of 5. Base **5** [ca. 1 g, prepared from 1.3 g (3.9 mmol) of HBr salt] in 30 mL of dry Et_2O was treated dropwise with 0.66 g (4.6 mmol) of MeI in 20 mL of dry Et_2O during 10 min at 0 °C. After the mixture was stirred overnight at room temperature, the precipitated **7** was collected and recrystallized from Me_2CO: yield 1.7 g (86%); mp 197–199 °C (colorless needles); NMR (Me_2SO-d_6) δ 3.20 and 3.32, (2 s, 6 H, NMe_2) 4.62 and 5.28 (2 s, 2 H, $C{=}CH_2$). Anal. ($C_{18}H_{26}INO$) C, H, N.

Hydrogenation of 7. PtO_2 (0.5 g), 2.0 g (5 mmol) of **7**, and 60 mL of 95% EtOH were shaken together at 25 °C and 30 psig for 12 h and then filtered. The filtrate was evaporated to dryness in vacuo to give 2.0 g of an oil, which was partitioned between 5% HCl and Et_2O. The Et_2O layer was extracted with 5% HCl. The combined aqueous acid layers were washed with Et_2O and made basic with NH_4OH. Et_2O extraction, drying (Na_2SO_4), and evaporation of the Et_2O gave 0.62 g (45%) of what appears to be compound **8**, 1-[2-(dimethylamino)ethyl]-1-(*m*-methoxyphenyl)-2-methylcyclohexane, resulting from absorption of 1 mol of H_2, Hofmann elimination, and absorption of a 2nd mol of H_2. The **HBr salt** of **8** (HBr gas–Me_2CO) crystallized from Me_2CO in prisms: mp 223–224 °C; NMR ($CDCl_3$) δ 0.65 (d, 3 H, *C*-Me), 2.24 and 2.30 (2 s, 6 H, NMe_2), 4.83 (s, 3 H, *O*-Me), 6.70 and 7.35 (m, 4 H, phenyl); EIMS, *m/e* 275. Anal. ($C_{18}H_{30}BrNO$) C, H, N.

Acknowledgment. We are indebted to Dr. Arthur E. Jacobson, National Institutes of Health, for arranging for C, H, and N analyses and mass spectral determinations and for hot-plate data. This research was supported by NIDA Grant DA-00490 and Contract 271-81-3830.

Registry No. (+)-**1**, 28623-81-6; (+)-**1**·HBr, 88588-34-5; (−)-**1**, 28623-84-9; ()-**1**·HBr, 53467-24-6; (±)-**3a**, 88550-29-2; (+)-**3a**, 88550-30-5; (+)-**3a**·HCl, 88550-31-6; (−)-**3a**, 88550-32-7; (−)-**3a**·HCl, 88550-33-8; **4**, 88550-34-9; **5**, 88550-35-0; **5**·HBr, 88550-36-1; **6**, 88550-37-2; **6**·HBr, 88550-38-3; **7**, 88550-39-4; **8**, 88550-40-7; **8**·HBr, 88550-41-8; methyltriphenylphosphonium iodide, 2065-66-9.

Studies on Heterocyclic Compounds. 6.[1] Synthesis and Analgesic and Antiinflammatory Activities of 3,4-Dimethylpyrano[2,3-*c*]pyrazol-6-one Derivatives

Sheng-Chu Kuo,*[†] Li-Jiau Huang,[†] and Hideo Nakamura[‡]

School of Pharmacy, China Medical College, Taichung 400, Taiwan, Republic of China, and Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33-94, 564 Suita/Osaka, Japan. *Received March 1, 1983*

A series of new 1- and 2-substituted 3,4-dimethylpyrano[2,3-*c*]pyrazol-6-one derivatives and 1-substituted 1,6-dihydro-4-methyl-6-oxopyrano[2,3-*c*]pyrazole-3-acetic acids were synthesized and examined for their analgesic and antiinflammatory activities. Most of these compounds showed more prominent analgesic activities than antiinflammatory activities, and this result was similar to that of aminopyrine. Among these compounds, 1,3,4-trimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one and 2,3,4-trimethylpyrano[2,3-*c*]pyrazol-6(2*H*)-one showed more potent analgesic activity than aminopyrine.

Recently, 3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (**1**) has been synthesized[1,2] and found to possess analgesic and antiinflammatory activities. However, neither the synthesis nor the biological activity of its N-substituted derivatives has been studied. We therefore carried out the synthesis of a series of N-substituted 3,4-dimethylpyrano[2,3-*c*]pyrazol-6-one derivatives in order to evaluate their biological activities. This report describes the synthetic results and the analgesic and antiinflammatory activities of these derivatives.

Chemistry. N-Alkylation of Compound 1. When compound **1** was treated with NaH in dry DMF, followed by reaction with methyl iodide at room temperature, two products (**3a** and **4a**) were obtained. The relative yield of the two products (**3a** and **4a**) was around 1:2. Based on mass spectra (M^+ *m/e* 178) and elemental analysis, the molecular formulas of both compounds were determined to be $C_9H_{10}N_2O_2$, which indicated that the two products could be isomers of *N*-methyl-3,4-dimethylpyrano[2,3-*c*]-pyrazol-6-ones. A similar result was realized when compound **1** was reacted with methyl iodide in dry DMF in the presence of K_2CO_3 under reflux.

The proposed isomeric products (**3a** and **4a**) could not be distinguished by the IR, UV, mass, and ^1H NMR spectral data (Table I). In order to ascertain the position of the *N*-methyl groups, the application of the method of $^1H\{^1H\}$ nuclear Overhauser effect in ^1H NMR spectroscopy was attempted. Unfortunately, no effect was observed upon the irradiation of the proton signals of the *N*-methyl protons [δ 3.80 (**3a**) and 3.85 (**4a**)]. We then investigated their ^{13}C NMR spectra. As shown in Table II, from the difference of chemical shifts between C-3 [δ 153.46 (**3a**), 150.67 (**4a**)] and C-9 [δ 151.07 (**3a**), 158.09 (**4a**)], the two structures could be tentatively assigned. The assignment was further confirmed by long-range coupling (J_{CNCHn} obtained by employing ^1H-gated decoupling method) between the protons of the *N*-methyl groups [δ 3.80 (**3a**), 3.85 (**4a**)] and C-9. The splittings of the signals of C-9 were quite different between those two isomers. The C-9 signal of compound **3a** is a quartet (J_{CNCH_3}) and that of **4a** is a

[†] China Medical College.
[‡] Dainippon Pharmaceutical Co., Ltd.

(1) Part 5: Kuo, S. C.; Lin, T. P.; Lin, L. D.; Hsu, H. Y.; Wu, C. H. *J. Nat. Prod.*, in press.
(2) (a) Huang, L. J.; Kuo, S. C.; Li, H. T. *J. Taiwan Pharm. Assoc.* **1979**, *31*, 47. (b) Renault, J.; Fauran, C.; Pellerin, F. *Bull. Soc. Chim. Fr.* **1963**, 2742. (c) Musante, C.; Fabbrini, L. *Farmaco, Ed. Sci.* **1953**, *8*, 264; *Chem. Abstr.* **1952**, *48*, 4536e. (d) Seidel, F.; Thier, W.; Uber, A.; Dittmer, *J. Chem. Ber.* **1935**, *68B*, 1913. (e) Yasunoba, S.; Sato, Y.; Shimeji, Y.; Kumakura, S.; Takagi, H. Japan Kokai 75 151 896, 1975; *Chem. Abstr.* **1976**, *84*, 16477*m*. (f) Khan, M. A.; Pagotto, M. C.; Ellis, G. P. *Heterocycles* **1977**, *6*, 983. (g) Khan, M. A.; Cosenza, A. G.; Ellis, G. P. *J. Heterocycl. Chem.* **1982**, *19*, 1077.

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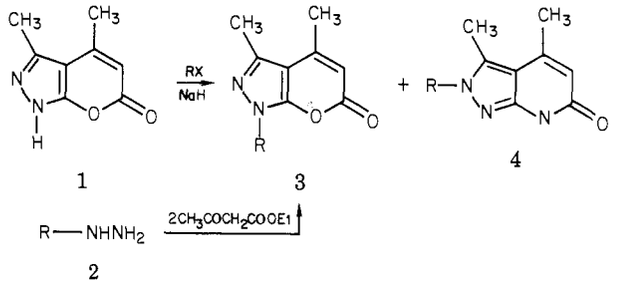
Scheme I[a]



[a] R = CH_3, C_2H_5, C_3H_7, $CH(CH_3)_2$, $(CH_2)_3CH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4CH_3$.

singlet. Based on the above analysis, the minor product (**3a**) was determined to be 1,3,4-trimethylpyrano[2,3-*c*]-pyrazol-6(1*H*)-one, and the major product (**4a**) was determined to be 2,3,4-trimethylpyranol[2,3-*c*]pyrazol-6-(2*H*)-one. These structural designations gained further support from the ^{13}C NMR studies of 1-ethyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (**3b**), 1-isopropyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (**3d**), 2-ethyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(2*H*)-one (**4b**), and 2-isopropyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(2*H*)-one **4d**). As shown in Table II, the chemical shifts of C-3 and C-9 of **3b,d** and **4b,d** were similar to those of **3a** and **4a**, respectively. The C-9 signals of **3b** and **3d** were a triplet (J_{CNCH_2R}) and doublet $J_{CNCH(R_2)}$, respectively, whereas the signals of **4b** and **4d** were singlets. Furthermore, compound **3a** could also be obtained by reacting methylhydrazine with ethyl acetoacetate.

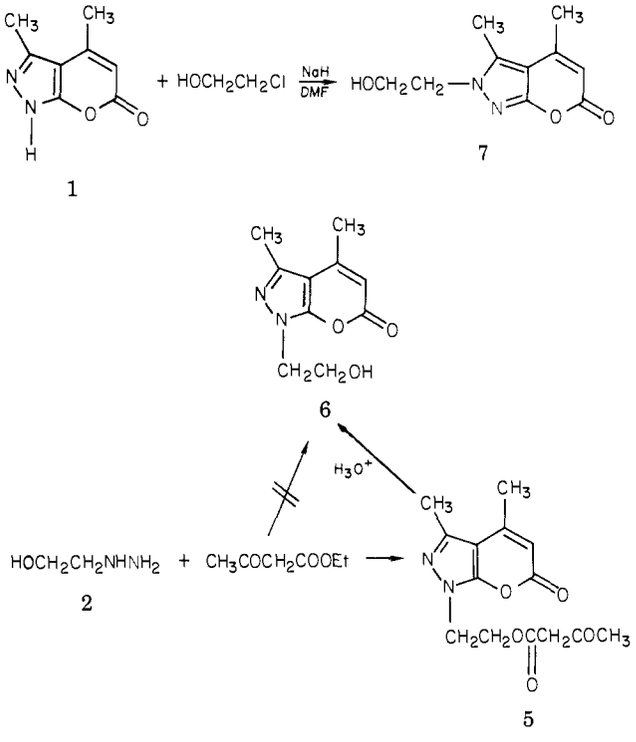
Compound **1** was then allowed to react with a variety of alkyl halides to afford the corresponding *N*-alkyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-ones (**3b–g** and **4b–g**). The N^1-alkyl and N^2-alkyl derivatives exhibited characteristic differences in their physical properties. It appeared reasonable that each pair of the compounds **3b–g** and **4b–g** could be differentiated with the values of their physical constants, such as R_f values, UV λ_{max} etc. (Table I). The above studies on the N-alkylation of 3,4-dimethylpyrano-[2,3-*c*]pyrazol-6-one (**1**) indicate that the nitrogen at the second position of this nucleus is more susceptible to alkylation than the nitrogen at the first position.

Formation of *N*-Aminoalkyl Derivatives of Compound 1. Direct condensation of (2,2-hydroxyethyl)-hydrazine with ethyl acetoacetate gave the 1-(2-acetoacetoxyethyl) derivative of **1** (**5**), which was then hydrolyzed with concentrated HCl to afford 1-(hydroxyethyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (**6**). 2-(Hydroxyethyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-(2*H*)-one (**7**) was obtained from the product mixture of the N-hydroxylation of compound **1** with 2-chloroethanol and NaH. Compound **7** was further transformed to 2-[2-(*N*,*N*-disubstituted-amino)ethyl]-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(2*H*)-ones (**9**) by chlorination with $SOCl_2$, followed by amination.

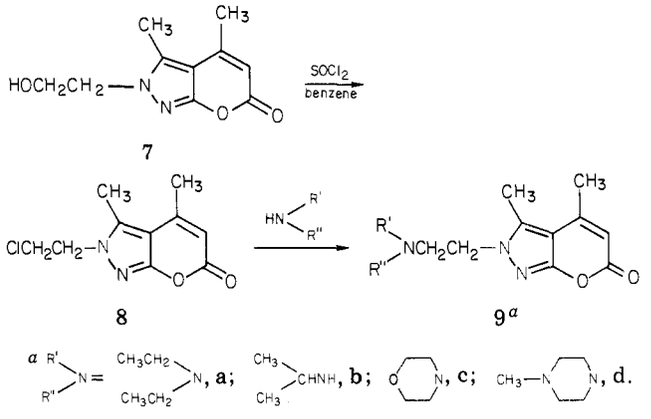
N-Acylation of Compound 1. Compound **1** was treated with a variety of acyl halides (or acyl anhydride) to afford the corresponding *N*-acyl-3,4-dimethylpyrano-[2,3-*c*]pyrazol-6-one (**10** or **11**), as shown in Scheme IV. The structures of the major products could not be assigned with certainty. However, by comparison of the R_f values with that of the trace products that were not isolated, we predict their structure to be the 2-acyl derivatives (**11**).

1-Substituted 1,6-Dihydro-4-methyl-6-oxopyrano-[2,3-*c*]pyrazole-3-acetic Acids (14). As shown in Table III, compound **3a** and **4a** showed more potent analgesic activity and weaker antiinflammatory effect than the

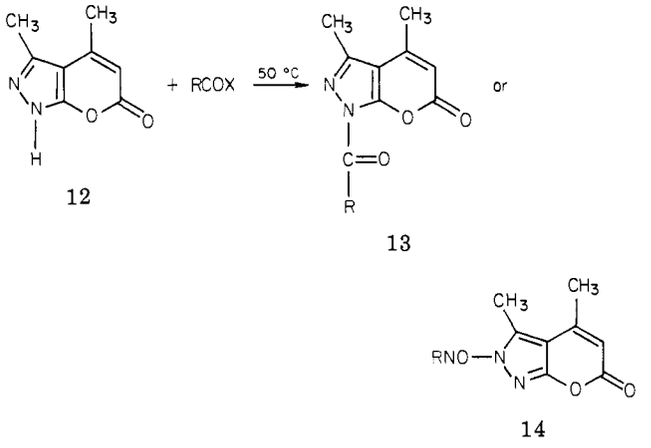
Scheme II



Scheme III



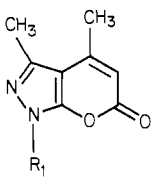
Scheme IV[a]



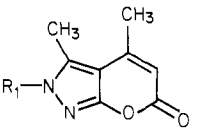
[a] R = H or CH_3.

standard drugs tested. Therefore, we attempted to replace the methyl group on C-3 with CH_2COOH. Thus, compound **12**, obtained from the reaction of diethyl 1,3-propanedicarboxylate with hydrazine or methylhydrazine,

Table I. 1-Substituted 3,4-Dimethylpyrano[2,3-*c*]pyrazol-6(2*H*)-ones (3) and 2-Substituted 1,4-Dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-ones (4)



3a-g

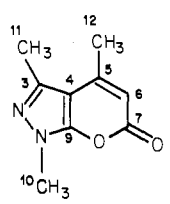


4a-g

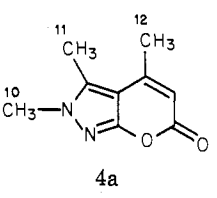
compd	R_1	mp, °C	yield,[a] %	TLC,[b] R_f	UV λ_{max} ($CHCl_3$), nm	NMR (in $CDCl_3$) chem shift, δ: CH_3 (3-position)	NMR (in $CDCl_3$) chem shift, δ: CH_3 (4-position)	formula[c]
3a	CH_3	171-172	30	0.70	314.5	2.36	2.33	$C_9H_{10}N_2O_2$
4a	CH_3	180-181	61	0.47	305	2.48	2.33	$C_9H_{10}N_2O_2$
3b	C_2H_5	111-113	28	0.82	314	2.43	2.35	$C_{10}H_{12}N_2O_2$
4b	C_2H_5	135-138	57	0.54	310	2.49	2.33	$C_{10}H_{12}N_2O_2$
3c	C_3H_7	81-83	30	0.86	313	2.41	2.36	$C_{11}H_{14}N_2O_2$
4c	C_3H_7	86-88	59	0.61	310	2.49	2.32	$C_{11}H_{14}N_2O_2$
3d	$CH(CH_3)_2$	104-105	28	0.87	320	2.39	2.33	$C_{11}H_{14}N_2O_2$
4d	$CH(CH_3)_2$	194-196	56	0.64	310	2.50	2.34	$C_{11}H_{14}N_2O_2$
3e	$(CH_2)_3CH_3$	61-63	27	0.89	313	2.39	2.34	$C_{12}H_{16}N_2O_2$
4e	$(CH_2)_3CH_3$	73.5-75	56	0.70	310	2.49	2.33	$C_{12}H_{16}N_2O_2$
3f	$CH_2CH(CH_3)_2$	96-98	27	0.88	319	2.43	2.40	$C_{12}H_{16}N_2O_2$
4f	$CH_2CH(CH_3)_2$	85-87	56	0.68	309	2.46	2.32	$C_{12}H_{16}N_2O_2$
3g	$(CH_2)_4CH_3$	66-68	28	0.90	313	2.39	2.33	$C_{13}H_{18}N_2O_2$
4g	$(CH_2)_4CH_3$	50-51	57	0.75	310	2.43	2.35	$C_{13}H_{18}N_2O_2$

[a] By method A of the N-alkylation of compound **2**. [b] Adsorbent: Wakogel B-5FM (silica gel); solvent: benzene/ether, 1:1. [c] Analyzed for C, H, N; analytical results were within ±0.4% of the theoretical value.

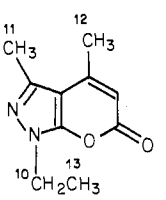
Table II. ^{13}C NMR of 3a-c and 4a-c in $CDCl_3$



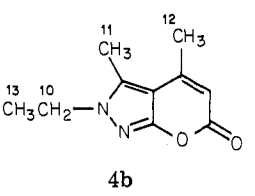
3a



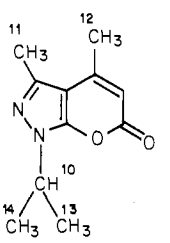
4a



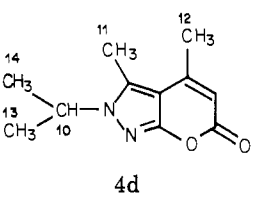
3b



4b



3d



4d

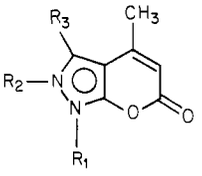
	^{13}C NMR chem shift, δ (multiplicity)[a]: 3a	4a	3b	4b	3d	4d
C-3	153.46 (s-q)	150.67 (s-q)	153.59 (s-q)	151.07 (s-q)	153.61 (s-q)	150.98 (s-q)
C-4	100.65 (s-m)	101.87 (s-m)	100.80 (s-m)	101.70 (s-m)	100.87 (s-m)	101.50 (s-m)
C-5	142.78 (s-dq)	135.74 (s-dq)	142.78 (s-dq)	134.99 (s-dq)	142.45 (s-dq)	134.31 (s-dq)
C-6	104.18 (d-dq)	108.45 (d-dq)	104.33 (d-dq)	108.12 (d-dq)	104.28 (d-dq)	108.34 (d-dq)
C-7	160.04 (s-d)	161.57 (s-d)	160.26 (s-d)	161.68 (s-d)	160.21 (s-d)	161.77 (s-d)
C-9	151.07 (s-q)	158.09 (s)	150.48 (s-t)	158.04 (s)	150.01 (s-d)	158.17 (s)
C-10	33.51 (q)	36.32 (q)	42.11 (t-tq)	44.17 (t-tq)	49.54 (d-m)	50.07 (d-m)
C-11	14.48 (q)	11.45 (q)	14.52 (q)	11.25 (q)	14.67 (q)	11.21 (q)
C-12	19.59 (q-qd)	19.15 (q-dq)	19.63 (q-dq)	19.30 (q-qd)	19.65 (q-qd)	19.48 (q-qd)
C-13			14.56 (q-qt)	15.11 (q-qt)	21.58 (q-m)	22.24 (q-m)
C-14					21.58 (q-m)	22.24 (q-m)

[a] J_{CH}, J_{CCH}, J_{CNCH}, or J_{CCCH} obtained by employing ^1H-gated decoupling method.

was treated with ethyl acetoacetate to afford the corresponding ethyl 1,6-dihydro-4-methyl-6-oxopyrano[2,3-*c*]-pyrazole-3-acetate (**13a**) or its 1-methyl derivative (**13b**). Hydrolysis of **13a,b** with concentrated HCl at 50 °C afforded the corresponding 1,6-dihydro-4-methyl-6-oxopyrano[2,3-*c*]pyrazole-3-acetic acids (**14a,b**).

Pharmacological Activity. The pharmacological activities of the compounds studied are summarized in Table III. Most compounds showed more prominent analgesic activity than antiinflammatory activity. Substitution with lower alkyl groups at R_1 and R_2 resulted in an increase in analgesic activity as compared with compound **1**, but the antiinflammatory activity was reduced. It is also clear that substituents larger than propyl groups tend to decrease analgesic activity. The order of the analgesic potency was $CH_3 > C_2H_5 > C_3H_7$ at both R_1 and R_2. The *N*-methyl-substituted derivatives (**3a** and **4a**) were analgesically the strongest among the compounds tested, and their potency was 2 to 3 times that of aminopyrine and more than 10 times that of aspirin. However, they, unlike the reference drugs tested, did not show antiinflammatory activity after oral administration of 80 mg/kg. The most active congener

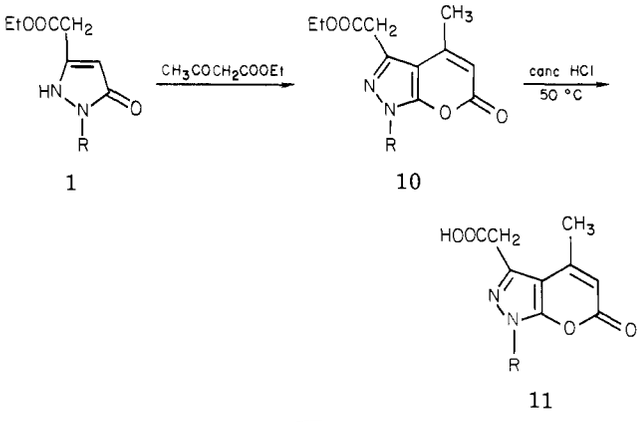
Table III. Analgesic and Antiinflammatory Activities



compd	R_1	R_2	R_3	analgesic[a] ED_{50}, mg/kg po (95% CL)	antiinflammatory inhibn,[b] %
1	H			40.6 (26.0-63.4), n = 15	23.0**
3a	CH_3		CH_3	11.6 (5.83-23.0), n = 30	4.7
4a		CH_3	CH_3	6.09 (2.83-13.1), n = 46	3.3
3b	C_2H_5		CH_3	24.9 (13.5-46.0), n = 22	12.1
4b		C_2H_5	CH_3	14.6 (6.19-34.3), n = 15	13.1*
3c	C_3H_7		CH_3	54.3 (30.3-97.1), n = 15	-0.6
4c		C_3H_7	CH_3	72.6 (45.8-115), n = 16	4.9
3d	$CH(CH_3)_2$		CH_3	⩾100	1.1
4d		$CH(CH_3)_2$	CH_3	35.3 (26.7-46.7), n = 18	3.7
3e	$(CH_2)_3CH_3$		CH_3	>100	-0.9
4e		$(CH_2)_3CH_3$	CH_3	>100	-3.1
3f	$(CH_2)_4CH_3$		CH_3	⩾100	3.5
4f		$(CH_2)_4CH_3$	CH_3	60.6 (20.2-182), n = 16	-5.4
6	CH_2CH_2OH		CH_3	100 (ca.)	-0.9
7		CH_2CH_2OH	CH_3	>100 (ca.)	1.8
8		CH_2CH_2Cl	CH_3	56.0 (14.7-214), n = 17	-2.1
9a		$CH_2CH_2N(C_2H_5)_2$	CH_3	100 (ca.)	17.0**
9c		CH_2CH_2-c-$N(CH_2CH_2)_2O$	CH_3	>100	4.4
11a		$COCH_3$	CH_3	12.2 (5.45-27.5), n = 31	4.5
11b		$COCH_2Cl$	CH_3	>100	5.2
11d		CO-C_6H_5	CH_3	35.0 (16.9-72.3), n = 24	11.9
11c			CH_3	74.3 (37.2-148), n = 15	2.2
14a	H		CH_2COOH	>100	6.4
13a	H		$CH_2COOC_2H_5$	>100	-4.1
14b	CH_3		CH_2COOH	>100	-3.1
13b	CH_3		$CH_2COOC_2H_5$	>100	-3.1
aminopyrine				19.2 (10.7-34.4), n = 32	17.5**
aspirin				>100	25.8**
ibuprofen				50.0 (32.7-76.2), n = 37	39.0**
mefenamic acid				45.9 (26.8-78.6), n = 19	33.1**[c]

[a] Phenylquinone writhing method in mice. [b] At 3 h after carrageenin injection in the hind-paw edema test in rats; dose = 80 mg/kg. * = $0.01 < p < 0.05$ and ** = $p < 0.01$ significantly different from the vehicle control. [c] 40 mg/kg po.

Scheme V[a]



1 10 11

[a] R = CH_3, $ClCH_2$,  ,  .

in antiinflammatory activity was compound **9a**, which was not as active as ibuprofen and mefenamic acid. Compounds **14a** and **14b** bearing the CH_2COOH group at R_3 lacked such activity. Thus, it appears that the structural requirement for maximum analgesic activity may not be the same as that for antiinflammatory activity, although there is some overlap.

The mice manifested a slight decrease in spontaneous motor activity, ptosis, and slight muscle relaxation after oral administration of compounds **3a** (500–1500 mg/kg) and **4a** (100–500 mg/kg) to female mice (24–26 g) of ddN strain, but hypnosis and Straub tail reaction did not occurred. The acute lethal toxicity (LD50 on day 7) was more than 1500 mg/kg and about 250 mg/kg for compounds **3a** and **4a**, respectively. Thus, the therapeutic index (LD50/analgesic ED50) of compounds **3a** and **4a** was about 5 and 1.5 times, respectively, larger than that of aminopyrine (LD50 = 523 mg/kg po). The pharmacological profile of compounds **3a** and **4a** and their related analogues was similar to aminopyrine rather than acidic nonsteroidal antiinflammatory drugs and narcotics.

The investigations of compounds **2**, **3a**, and **4a** on 5-hydroxytryptamine release from rat spinal slices are still in progress, and the results will be published later.

Further structural modification and quantitative QSAR analysis are needed to establish the optimal structural requirements of these compounds.

Experimental Section

Melting points were determined on a Büchi melting point apparatus, in open capillary tubes, and are uncorrected. Elemental analyses were performed by Kyoto University (Japan) and Chung Shan Institute of Science Technology (Republic of China) and were within 0.4% of the calculated values. IR spectra were

recorded on SHIMAZU IR-440. NMR spectra were determined in the indicated solvent on a JEOL PS-100 with tetramethylsilane as internal standard for proton spectra. Chemical shifts are given in δ units, and coupling constants are in hertz. Splitting patterns are designated as follow: s, singlet; br, broad; d, doublet; t, triplet; q, quartet; m, multiplet. ^{13}C NMR spectra were obtained on a JEOL FX-100. Mass spectra were recorded on a Hitachi RMU 6C. UV spectra were recorded on a SHIMAZU UV-210A.

N-Alkylation of 3,4-Dimethylpyrano[2,3-*c*]pyrazol-6-one (1). Method A. 3,4-Dimethylpyrano[2,3-*c*]pyrazol-6-one (1; 4.0 g, 0.025 mol) was dissolved in dry DMF (40 mL), and NaH (50% in oil; 1.2 g, 0.05 mol) was added portionwise with stirring for 30 min at room temperature. Alkyl halide (0.025 mol) was then added dropwise at 30–40 °C. Stirring was continued for an additional 30 min, and then the reaction mixture was poured into ice–water and extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography (benzene–silica gel) to give **3a–g** and **4a–g** (Table I).

Method B. Compound 1 (4.0 g, 0.025 mol) was dissolved in dry DMF (40 mL), and K_2CO_3 (3.5 g, 0.025 mol) was added. The alkyl halide (0.025 mol) was then added dropwise under reflux. The mixture was heat at reflux for an additional 1 h. After the reaction was completed, the solvent was removed by evaporation. After the reaction was completed, the solvent was removed by evaporation. Subsequent workup was performed as in method A, and the same result was obtained.

Reaction of Alkylhydrazines (2) with Ethyl Acetoacetate. Ethyl acetoacetate (13.0 g, 0.1 mol) was heated at 140 °C, and the alkylhydrazines (0.01 mol) were added dropwise with stirring. Water and ethanol that formed in the reaction were distilled off, and the mixture was heated under reflux. Upon completion of the reaction, the mixture was allowed to stand at room temperature; the solid thus separated was collected and recrystallized from ethanol to afford **3a–g** (Table I).

1-(2-Hydroxyethyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-(1*H*)-one (6) and 2-(2-Hydroxyethyl)-3,4-dimethylpyrano-[2,3-*c*]pyrazol-6(2*H*)-one (7). Method A. Compound 1 (4.0 g, 0.025 mol) and 2-chloroethanol (2.0 g, 0.025 mol) were allowed to react as in method A of the N-alkylation of compound 1 described above to give compounds **6** (0.5 g, 10%) and **7**. Compound **6**: yield 0.5 g (10%); mp 158–160 °C; R_f 0.4 (Wakogel B-5 FM–EtOAc); IR (KBr) ν_{max} 3500–2500 (OH), 1720 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.72 (m, 1 H, C-5 H), 4.74 (t, 1 H, OH), 3.98 (t, 2 H, CH_2OH), 3.72 (t, 2 H, NCH_2), 2.36 (s, 6 H, 2 CH_3); mass spectrum, *m/e* 208 (M^+). Anal. ($C_{10}H_{12}N_2O_3$) C, H, N. Compound **7**: yield 1.8 g (35%); mp 176–178 °C; R_f 0.29 (Wakogel B-5 FM–EtOAc); IR (KBr) ν_{max} 3500–2500 (OH), 1720 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.72 (m, 1 H, C-5 H), 4.83 (t, 1 H, OH), 4.08 (t, 2 H, CH_2OH), 3.71 (t, 2 H, $>NCH_2$), 2.64 (s, 3 H, C-4 CH_3), 2.41 (s, 3 H, C-3 CH_3); mass spectrum, *m/e* 208 (M^+). Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

Method B. Ethyl acetoacetate (13.0 g, 0.1 mol) and (2-hydroxyethyl)hydrazine (0.8 g, 0.01 mol) were allowed to react as in the method of the reaction of alkylhydrazine (**2**) with ethyl acetoacetate, to afford 1-(2-acetoacetoxyethyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (**5**): yield 2.3 g (80%); mp 120–122 °C; IR (KBr) ν_{max} 1720, 1700 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.70 (m, 1 H, C-5 H), 4.20–4.50 (m, 4 H, CH_2CH_2), 3.40 [s, 2 H, $C(=O)CH_2C(=O)$], 2.36 (s, 6 H, C-3 and C-4 CH_3), 2.30 [s, 3 H, $C(=O)CH_3$]; mass spectrum, *m/e* 229 (M^+). Anal. ($C_{14}H_{16}N_2O_5$) C, H, N.

Compound **5** (3 g, 0.01 mol) was then dissolved in concentrated HCl (30 mL) and stirred at 50 °C for 1 h. After the reaction was completed, the solvent was removed by evaporation. The residue was washed with water and recrystallized from ethanol to give the compound **6**. This product was confirmed by mixture melting point and comparison of its IR spectra with that of a sample obtained from the method A.

2-(2-Chloroethyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-(1*H*)-one (8). To a suspension of **7** (0.5 g, 0.002 mol) in dry benzene (80 mL) was added dropwise $SOCl_2$ (10 mL). The mixture was heated at reflux for 72 h. After the reaction was completed, the solvent was removed by evaporation. The residue was washed with water and recrystallized from benzene–CCl_4 to give **8**: yield 0.4 g (90%); mp 127–128 °C; IR (KBr) ν_{max} 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.72 (m, 1 H, C-5 H), 4.34 (t, 2 H, CH_2Cl), 3.91 (t, 2 H, $>NCH_2$), 2.56 (s, 3 H, C-4 CH_3), 2.38 (s, 3 H, C-3 CH_3); mass spectrum, *m/e* 226 (M^+). Anal. ($C_{10}H_{11}ClN_2O_2$) C, H, N.

2-(2-Aminoethyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-(2*H*)-one (9a–d). Compound **8** (0.5 g, 0.002 mol) was dissolved in dry DMF (10 mL), and an amine (0.004 mol) was added with stirring at room temperature. The mixture was then heated at reflux for 24 h. After the reaction was completed, the solvent was removed by evaporation to leave a residue, which was dissolved in $CHCl_3$ and washed with water. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (EtOAc–silica gel) to give **9a–d** (Table I).

1-Acetyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (10a) or 2-Acetyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(2*H*)-one (11a). To a suspension of compound 1 (2 g, 0.013 mol) in dry benzene (150 mL) was added dropwise AC_2O (2.0 g, 0.02 mol). The reaction mixture was heated at reflux for 5 h and then allowed to stand at 30 °C for 1 h. The solid thus separated was collected and recrystallized from benzene–MeOH to afford **10a** or **11a**, mp 185–187 °C. The filtrate was evaporated, and the residue was purified by column chromatography (benzene–silica gel) to give additional **10a** or **11a**: total yield 1.2 g (47%); IR (KBr) ν_{max} 1700, 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.90 (m, 1 H, C-5 H), 2.80 [s, 3 H, $C(=O)CH_3$], 2.62 (s, 3 H, C-4 CH_3), 2.40 (s, 3 H, C-3 CH_3); mass spectrum, *m/e* 194 (M^+). Anal. ($C_{10}H_{10}N_2O_3$) C, H, N.

1-(Chloroacetyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-(1*H*)-one (10b) or 2-(Chloroacetyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(2*H*)-one (11b). Compound 1 (2 g, 0.013 mol) and chloroacetyl chloride (10 g, 0.13 mol) were reacted as in the method of preparing **10a** or **11a** to afford **10b** or **11b**: yield 2.9 g (98%); mp 198–200 °C. Anal. ($C_{10}H_9ClN_2O_3$) C, H, N.

1-(5-Furoyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (10c) or 2-(5-Furoyl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-(2*H*)-one (11c). Compound 1 (2 g, 0.013 mol) and furoyl chloride (3.1 g, 0.04 mol) were reacted as in the method of preparing **10a** or **11a** to afford **10c** or **11c**: yield 1.4 g (45%); mp 241–243 °C. Anal. ($C_{13}H_{10}N_2O_4$) C, H, N.

1-Benzoyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (10d) or 2-Benzoyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-(2*H*)-one (11d). Compound 1 (2 g, 0.013 mol) and benzoyl chloride (3.6 g, 0.026 mol) were reacted as in the method of preparing **10a** or **11a** to afford **10d** or **11d**: yield 1.7 g (48%); mp 178–181 °C. Anal. ($C_{15}H_{12}N_2O_3$) C, H, N.

Ethyl 5-Oxo-3-pyrazolineacetate (12a). Diethyl 1,3-propanedicarboxylate (20 g, 0.1 mol) was stirred at room temperature, and hydrazine hydrate (5 g, 0.1 mol) was added dropwise. The reaction mixture was heated at reflux for 2 h; then the crystals that separated were collected and recrystallized from benzene to afford **12a**: 16.1 g (95%); mp 115–116 °C. Anal. ($C_7H_{10}N_2O_3$) C, H, N.

Ethyl 1-Methyl-5-oxo-3-pyrazoline-3-acetate (12b). Diethyl 1,3-propanedicarboxylate (20 g, 0.01 mol) and methylhydrazine (4.6 g, 0.1 mol) were reacted as in the method of preparing **12a** to afford **12b**: yield 16.7 g (91%); mp 90–92 °C. Anal. ($C_8H_{12}N_2O_3$) C, H, N.

Ethyl 1,6-Dihydro-4-methyl-6-oxopyrano[2,3-*c*]pyrazole-3-acetate (13a). Compound **12a** (20 g, 0.11 mol) and ethyl acetoacetate (71 g, 0.55 mol) were allowed to react as in the preparation of compound **5** to afford **13a**: yield 20.7 g (80%); mp 151–153 °C; IR (KBr) ν_{max} 1700, 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.90 (m, 1 H, C-5 H), 4.25 (q, 2 H, CH_2CH_3), 4.00 [s, 2 H, $CH_2C(=O)OR$], 2.34 (s, 3 H, C-4 CH_3), 1.28 (t, 3 H, CH_2CH_3); mass spectrum, *m/e* 236 (M^+). Anal. ($C_{11}H_{12}N_2O_4$) C, H, N.

Ethyl 1,6-Dihydro-1,4-dimethyl-6-oxopyrano[2,3-*c*]-pyrazole-3-acetate (13b). Compound **12b** (4 g, 0.02 mol) and ethyl acetoacetate (26 g, 0.20 mol) were allowed to react as in the preparation of compound **5** to afford **13b**: yield 3.7 g (75%); mp 140–142 °C. Anal. ($C_{12}H_{14}N_2O_4$) C, H, N.

1,6-Dihydro-4-methyl-6-oxopyrano[2,3-*c*]pyrazole-3-acetic Acid (14a). Compound **13a** (2.3 g, 0.01 mol) was dissolved in concentrated HCl (100 mL) and stirred at 50 °C for 1 h. The solvent was evaporated under 50 °C. The residue was washed with water and recrystallized from benzene to give **14a**: yield 1.5 g (72%); mp 245–246 °C dec; IR (KBr) 2900–2500 (OH), 1720 (C=O) cm^{-1}. Anal. ($C_9H_8N_2O_4$) C, H, N.

1,4-Dimethylpyrano[2,3-*c*]pyrazole-3-acetic Acid (14b). Compound **13b** (2.5 g, 0.01 mol) was treated as in the preparation of **14a**, to afford **14b**: yield 1.7 g (80%); mp 213–214 °C dec. Anal. ($C_{10}H_{10}N_2O_4$) C, H, N.

Analgesic Assay.[3,4] Phenylquinone writhing was induced by phenylquinone (0.03% in 5% ethanol aqueous solution), 10 mL/kg, ip, in female mice (18–22 g) of ddN strain. The number of writhes was counted for 15 min, beginning 5 min after phenylquinone injection. Each compound was administered orally 30 min before phenylquinone. Five to ten mice were used for each dose.

Antiinflammatory Activity.[5] Hind-paw edema was induced by a subcutaneous injection of 0.1 mL of a 1% carrageenin solution into the hind foot pad of male rats (90–120 g) of Wistar strain. Each compound was administered orally 1 h before carrageenin injection. Five to ten rats were used for each dose.

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Registry No. 1, 5203-98-5; **2** (R = CH_3), 60-34-4; **2** (R = C_2H_5), 624-80-6; **2** (R = C_3H_7), 5039-61-2; **2** [R = $(CH(CH_3)_2]$], 2257-52-5; **2** [R = $(CH_2)_3CH_3$], 3530-11-8; **2** [R = $CH_2CH(CH_3)_2$], 42504-87-0; **2** [R = $(CH_2)_4CH_3$], 2656-71-5; **3a**, 5775-94-0; **3b**, 88549-98-8; **3c**, 88549-99-9; **3d**, 88550-00-9; **3e**, 88550-01-0; **3f**, 88550-02-1; **4a**, 87343-65-5; **4b**, 88563-11-5; **4c**, 88550-03-2; **4d**, 88550-04-3; **4e**, 88550-05-4; **4f**, 88550-06-5; **5**, 88550-07-6; **6**, 67056-25-1; **7**, 88550-08-7; **8**, 88550-09-8; **9a**, 88550-10-1; **9c**, 88550-11-2; **11a**, 88550-12-3; **11b**, 88550-13-4; **11c**, 88550-14-5; **11d**, 88550-15-6; **12a**, 88550-16-7; **12b**, 88563-12-6; **13a**, 64518-00-9; **13b**, 64518-02-1; **14a**, 88550-17-8; **14b**, 88550-18-9; 2-furoyl chloride, 527-69-5; 2-chloroethanol, 107-07-3; (2-hydroxyethyl)hydrazine, 109-84-2; chloroacetyl chloride, 79-04-9; benzoyl chloride, 98-88-4; $EtOCOCH_2COCH_2COOEt$, 105-50-0; CH_3COCH_2COOEt, 141-97-9.

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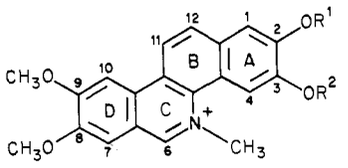
Synthesis and Biological Activity of Structural Analogues of the Anticancer Benzophenanthridine Alkaloid Nitidine Chloride

Mark Cushman,* Prem Mohan, and Edward C. R. Smith

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. *Received June 24, 1983*

The indenoisoquinoline analogue **9** of nitidine (**1**) has been prepared and found to possess significant anticancer activity against L1210 lymphoid leukemia, P388 lymphocytic leukemia, and B16 melanocarcinoma. Analogue **14**, which lacks the B ring of nitidine (**1**), has also been synthesized. Compound **14** retains the in vitro toxicity associated with nitidine (**1**) but is devoid of antileukemic activity. The structural factors that may contribute to the difference in biological activity between the two closely related analogues **9** and **14** are discussed.

Nitidine (**1**) and fagaronine (**2**) are benzophenanthridine



1, $R^1 + R^2 = CH_2$
2, $R^1 = H$, $R^2 = CH_3$

alkaloids that have been isolated from *Zanthoxylum nitidum*[1,2] and *Fagara zanthoxyloides*,[3] respectively. The structure of nitidine (**1**) was established by its conversion to known compounds[2] and by the synthesis of dihydronitidine,[4,5] while the structure of fagaronine (**2**) was originally proposed on the basis of spectral evidence[3,6] and was later confirmed by total sythesis.[7] Several nitidine (**1**) syntheses have also been performed.[4,5,8]

Both nitidine (**1**) and fagaronine (**2**) have displayed activity against the mouse leukemia L1210 and P388 systems.[3,9,10] Nitidine (**1**) has also shown curative activity against Lewis lung carcinoma.[9b] The anticancer activity of these compounds and related benzophenanthridine alkaloids has been correlated with inhibition of reverse transcriptase activity of RNA tumor viruses by binding to the A-T base pairs of the template primers,[11] inhibition of transfer RNA methyltransferase,[12] and the iminium ion

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