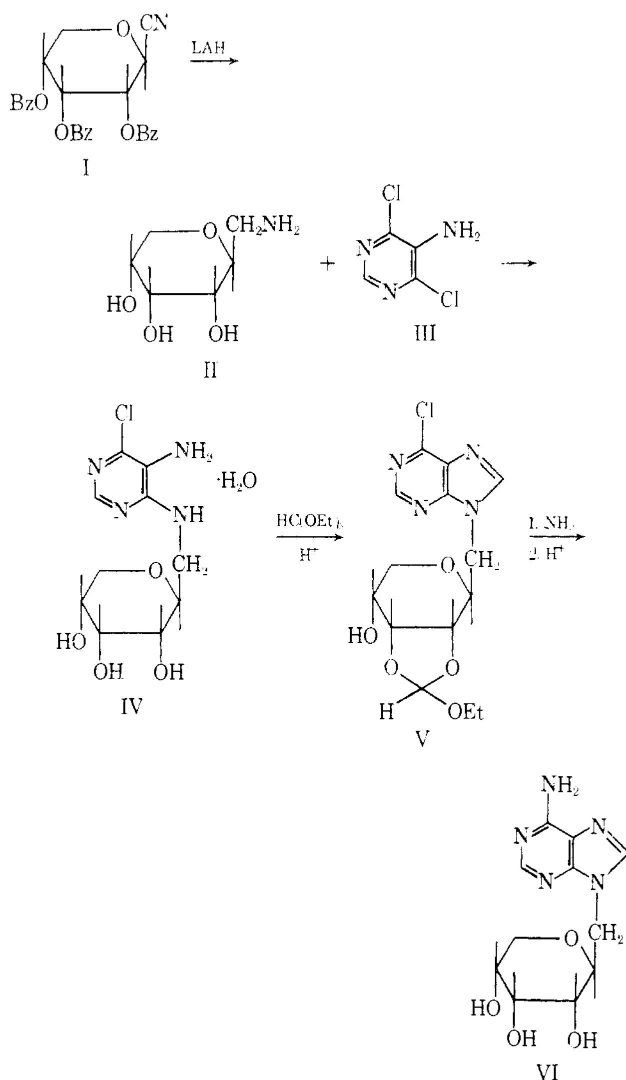

performed by incorporating the compound into a suitable agar at the concentration of 500 ppm. After solidification, droplets of the test organisms were applied to the surface. The plates were incubated. Observations for kill or inhibition were made after a suitable time.

The only activity observed for VI for the organisms tested was somewhat less than 100% inhibition at 500 ppm against *Mycobacterium phlei*. The other organisms tested were *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli*, *Trichophyton mentagrophytes*, *Bacillus subtilis*, *Aerobacter aerogenes*, *Candida pelliculosa*, *Pseudomonas sp.* Strain 10, *Salmonella typhosa*, *Pullularia pullulans*, *Pseudomonas aeruginosa*, *Aspergillus terreus*, *Rhizopus nigricans*.

Experimental Section[8]

Reagents and Assay Procedure.—Adenosine and adenosine deaminase were purchased from the Sigma Chemical Co. The general method of assay has been described by Kaplan[9] and involves measuring the rate of disappearance of the absorption band of adenosine at 265 mμ. All enzymatic reactions were performed in 0.05 *M* phosphate buffer at pH 7.6 and 25°. The substrate and the stock solutions of all reagents were prepared in 0.05 *M* phosphate buffer at pH 7.6. For the assay, the cell contained a total volume of 3.1 ml which was 0.066 m*M* with respect to adenosine. To study inhibition, appropriate amounts of buffer were excluded from the cells and were replaced by an equal volume of a solution of the inhibitor in phosphate buffer.

6-(5-Amino-6-chloro-4-pyrimidinylamino)-1,5-anhydro-6-deoxy-L-allitol (IV).—A suspension of 28.0 g (59.4 mmoles) of I[5] in 300 ml of THF was slowly added to a mixture of $LiAlH_4$ (13.3 g) in 150 ml of THF. The mixture was stirred at room temperature for 1 hr and heated under reflux for 1.5 hr. The excess hydride was destroyed by the addition of EtOH, H_2O, and NH_4OH. The mixture was filtered through Filtered and the filtrate was passed through a column (4.7 × 56 cm) of Dowex 50W-X8 resin. The column was eluted (H_2O, 3000 ml, and then with 2 *N* NH_4OH, 2000 ml). Concentration of the NH_4OH *in vacuo* gave II as a light brown syrup, yield 3.98 g. Compound II was identified by converting a small amount to the known hydrochloride salt.[5]

A mixture of 2.63 g (16.2 mmoles) of unpurified II, 2.42 g (16.2 mmoles) of 5-amino-4,6-dichloropyrimidine (III), and 1.70 g (32.4 mmoles) of NEt_3 in 50 ml of *n*-BuOH was heated under reflux for 20 hr. The reaction mixture was evaporated *in vacuo* and the tan oil was crystallized (H_2O). The white solid was removed by filtration; yield 3.793 g (84%), mp 159–161°. Recrystallization (H_2O) gave 2.30 g of pure product, mp 174–176°, $[\alpha]^{25}D$ −18.4 ± 0.3° (0.1 *N* HCl). *Anal.*[10] ($C_{10}H_{15}N_4ClO_4 \cdot H_2O$) C, H, N.

6-(6-Amino-9-purinyl)-1,5-anhydro-6-deoxy-L-allitol (VI).—To a suspension of 2.30 g (7.48 mmoles) of IV in 25 ml of ethyl orthoformate was added 10.3 mmoles of concentrated HCl. The mixture was stirred overnight at room temperature and evaporated *in vacuo* to a clear oil (V). The unpurified ortho ester was dissolved in 30 ml of liquid NH_3 and heated in a steel bomb at 55–60° for 18 hr. The volatile materials were evaporated and the tan solid was dissolved in 7 ml of 5% HCl and allowed to stand at room temperature for 30 min. The acidic solution was made basic with concentrated NH_4OH and chilled. A white solid was collected by filtration and gave 1.78 g (85%) of VI, mp 248–252°. One recrystallization (H_2O) gave the pure product as a monohydrate, mp 258–259°, $\lambda_{max}^{pH\ 7}$ 261 mμ (ε 1.41 × 10^4), $[\alpha]^{25}D$ −13.25 ± 0.3° (H_2O).

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(8) The ir spectra were determined on a Perkin-Elmer Model 137 spectrophotometer; the uv spectra were determined on a Perkin-Elmer Model 4000A spectrophotometer; the enzyme studies were done on a Gilford Model 2000 spectrophotometer. The optical rotations were taken on a Perkin-Elmer Model 141 polarimeter. Melting points were taken in open capillary tubes on a Mel-Temp apparatus and are uncorrected.

(9) N. O. Kaplan, *Methods Enzymol.*, **2**, 473 (1955).

(10) The analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values.

1-Acylindoles. IX. Syntheses of 1-Cinnamoyl-5-methoxy-2-methyl-3-indolylaliphatic Acids as Potential Antiinflammatory Agents

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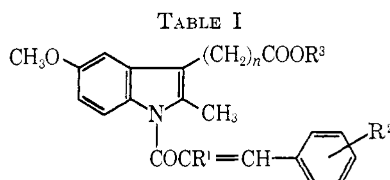
Recent interest in the antiinflammatory action of indomethacin prompted us to prepare 1-acyl-3-indolylaliphatic acids for antiinflammatory tests. Various kinds of compounds had been synthesized previously,

TABLE I



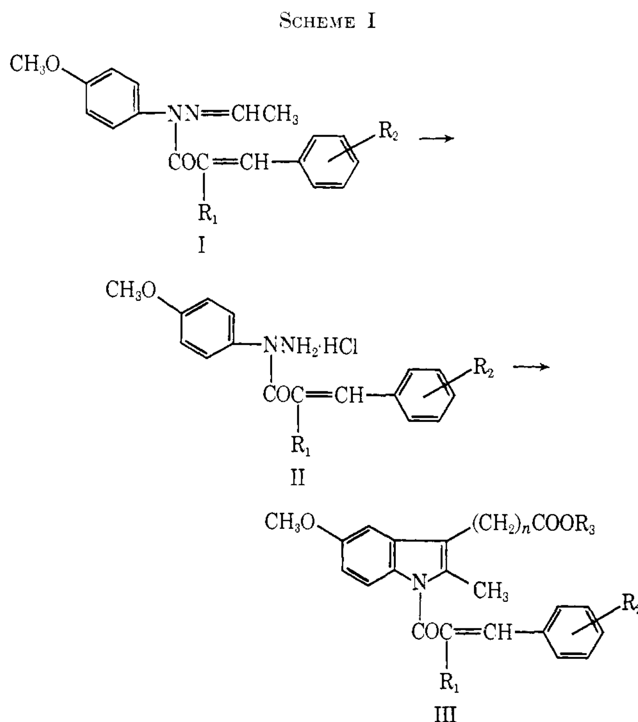
Compd	R	R²	R³	n	Prepn method	Yield, %[c]	Mp, °C	Formula[a,b]
1	H	H	H	1	A	50	170–171	$C_{21}H_{19}NO_4$
2	H	p-OCH_3	H	1	A	25	193–195	$C_{22}H_{21}NO_5$
3	H	p-Cl	H	1	A	67	220–221	$C_{21}H_{18}ClNO_4$
4	H	p-CH_3	H	1	A	37	195	$C_{22}H_{21}NO_4$
5	H	m-NO_2	H	1	A	. . .	203–204	$C_{21}H_{18}N_2O_6$
6	H	H	C_2H_5	1	D	25	68–69	$C_{23}H_{23}NO_4$
7	H	H	CH_3	1	C	24	86–86.5	$C_{22}H_{21}NO_4$
8	H	H	H	2	B	30	189–190	$C_{22}H_{21}NO_4$
9	H	H	H	3	B	28	125–126	$C_{23}H_{23}NO_4$
10	CH_3	H	H	1	A	26	153–154	$C_{22}H_{21}NO_4$
11	C_6H_5	H	H	1	A	16	174–175	$C_{27}H_{23}NO_4$

[a] The ir spectra of all compounds were as expected. [b] All compounds were analyzed for C, H, N. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [c] From acetaldehyde p-methoxyphenylhydrazone.

and 1-cinnamoyl-5-methoxy-2-methyl-3-indolylaliphatic acids have now been prepared.

The 1-cinnamoyl-5-methoxy-2-methyl-3-indolylaliphatic acid derivatives are prepared from acetaldehyde p-methoxyphenylhydrazone according to a novel procedure[1] as shown in Scheme I.

SCHEME I



First, N[1] acylation of an acetaldehyde p-methoxyphenylhydrazone with a cinnamoyl chloride gives an acetaldehyde cinnamoylphenylhydrazone (I). The unsymmetrical N[1]-cinnamoyl-p-methoxyphenylhydrazine hydrochloride (II) is prepared by hydrolysis of an appropriate I with EtOH–HCl. The 1-cinnamoyl-5-methoxy-2-methyl-3-indolylaliphatic acid (III) is obtained by treating an appropriate compound II with the requisite ketoaliphatic acid according to a Fischer indole reaction (Table I).

Pharmacological Results.—Compounds **1**, **6**, and **7** showed notably inhibitory action for carrageenin-induced edema with minimum toxicity. Especially, **1** inhibited the edema by 30% even at 2.5 mg/kg, while no toxic symptoms were observed after oral administration of 200 mg/kg in this animal test.

However, a ring substituent of a cinnamoyl group lowered the potency. Compounds **2–5** show relatively poor activity for inhibiting the edema as compared to the parent compound. The substitution of a 3-indolyl group at the α position of an aliphatic acid is effective, while with the same substitution at the β or γ positions the intrinsic activity seems to be lost (**8**, **9**). Methyl or phenyl substitution of the β position of a cinnamoyl group causes lowering of the potency (Table II).

Experimental Section

Melting points are uncorrected and were determined in open capillary tubes. Ir spectra were taken on a Shimazu 27G spectrophotometer.

1-Cinnamoyl-5-methoxy-2-methyl-3-indolylaliphatic Acids (III). Method A.—To a solution of 0.2 mole of acetaldehyde p-methoxyphenylhydrazone and 0.24 mole of pyridine in Et_2O (250 ml) was added dropwise 0.24 mole of a cinnamoyl chloride over 30 min below 5° with stirring. Stirring was continued for additional 3 hr with cooling. The precipitate was filtered off and washed (H_2O) to give an acetaldehyde cinnamoylphenylhydrazone (I). It was suspended in EtOH and treated with excess gaseous HCl with cooling. The mixture was allowed to stand in a refrigerator overnight, and the resultant crystals were filtered off to give an N[1]-cinnamoyl-p-methoxyphenylhydrazine hydrochloride (II). This compound (5 g) was heated with 15 g of levulinic acid at 70–80° with stirring. After cooling, the reaction mixture was poured into 100 ml of cold H_2O, and the resultant precipitate was filtered and washed (H_2O). Two recrystallizations (Me_2CO–H_2O) gave III.

Method B.—A N[1]-cinnamoyl-p-methoxyphenylhydrazone hydrochloride (II) (0.01 mole) prepared by method A, 0.013 mole of a ketoaliphatic acid, and 15 ml of AcOH were heated at 75° for 2 hr with stirring. After cooling, 50 ml of H_2O was added and the precipitate was filtered and washed (H_2O). Recrystallization (Me_2CO) gave III.

Methyl 1-Cinnamoyl-5-methoxy-2-methyl-3-indolylacetate (7). Method C.—A mixture of 3.0 g of N[1]-cinnamoyl-p-methoxyphenylhydrazine hydrochloride, 2.0 g of methyl levulinate, and

(1) H. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 17 (1968).

TABLE II

ANTIINFLAMMATORY ACTIVITY[a]

No.	Dose,[b] mg/kg	Inhib of edema[c]	Toxicity[d]
1	2.5	27.8	−
	10	46.0	−
	50	54.4	−
	200	67.1	−
2	200	8.0	−
3	200	19.7	−
4	200	0	−
5	200	5.5	−
6	50	41.2	−
	100	52.9	−
	200	68.8	+
7	50	30.2	−
	100	44.6	−
	200	70.9	−
8	200	8.7	−
9	200	0	−
10	200	33.0	+++
11	200	19.7	−
Indomethacin	2.5	31.3	−
	10	56.6	++
	20	57.5	+++
Phenylbutazone	50	30.4	−
	100	40.6	−

[a] Antiinflammatory activity was evaluated by the inhibitory effect on rat paw edema induced by injection of 0.05 ml of 1% carrageenin in sterile 0.9% NaCl.[2] [b] Test compounds were administered orally 1 hr before the injection of carrageenin. At each dose level, three to six rats were used. [c] Foot volume was measured at 3, 4, and 5 hr after the carrageenin injection and the mean of three measurements was calculated in each rat. Inhibition of edema is expressed as (1 − T/C) × 100, where T is mean edema volume of treated group and C is the mean volume of control group. [d] −, no blood in feces, body weight gain normal; +, no blood in feces, body weight decreased; ++, blood in feces, body weight decreased; +++, symptoms of ++ but some animals died during the 4 days after administration.

6 ml of AcOH was heated at 90–100° for 1 hr. After cooling, the reaction mixture was poured into 600 ml of cold H_2O. A resultant oily substance was extracted (Et_2O), and the ethereal layer was washed (H_2O) and dried (Na_2SO_4). The solvent was removed by distillation to give 3.2 g of an oily residue, which was chromatographed on silica gel and eluted with $CHCl_3$. Recrystallization (MeOH) gave 1.1 g (31%) of yellow needles of ester, mp 86–86.5°.

Ethyl 1-cinnamoyl-5-methoxy-2-methyl-3-indolylacetate (6) was prepared analogously, mp 68–69° (from EtOH–H_2O).

Pharmacological Tests.—Antiinflammatory activity of these compounds was tested in carrageenin-induced foot edema of rats.[2] Test materials were suspended in 0.5% solution of sodium carboxymethylcellulose and given by stomach tube 1 hr before the injection of carrageenin. For toxicity tests of these compounds, blood in feces was determined the day after the carrageenin test and the body weight of each rat was recorded daily for the following 4 days. These results are expressed as percentage of the inhibition in Table II.

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(2) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exptl. Biol. Med.*, **111**, 544 (1962).

Absolute Configuration of Some Benzomorphan Analgetics and Related Compounds

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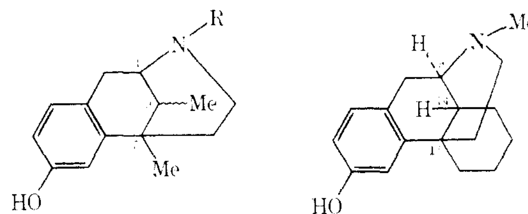
The analgetic potency of several 6,7-benzomorphan derivatives **1** is influenced both by the relative con-



figurations of the 5,9-dimethyl substituents (β diastereoisomers are more potent than α forms) and by absolute configuration within a particular enantiomorphic pair (in all cases examined, the activity of both α and β racemates largely resides in the *levo* antipode).[1,2] In related derivatives that are analgetic antagonists (*e.g.*, **1c** and **d**), activity differences between (±) diastereoisomers are insignificant, but pronounced potency variations among enantiomers are still found.[3] The relative 5,9-dialkyl configurations of several benzomorphan diastereoisomers are known (*α-cis* and *β-trans* with respect to the hydroaromatic ring) from rates of quaternization and pmr data,[4] and a recent X-ray analysis of the α-N-allyl derivative (**1f**)[5] supports these assignments. Knowledge of absolute configuration is confined to the α-(−)-5,9-diethyl analog of **1a** which has been shown to share common geometry with (−)-morphine through its synthesis from an intermediate derived from natural thebaine,[6] although there is evidence by the method of stereoselective adsorbents[7] that α-(−)-metazocine (**1a**) and phenazocine (**1b**) are both related to morphine. Ord data are now presented which (along with some chemical transformations) firmly establish the absolute configurations of benzomorphan enantiomers of both the α and β type.

Numerical characteristics of ord curves recorded for α-(−)- and β-(+)-**1a** (metazocine), levorphanol, and related compounds in EtOH or H_2O are shown in Table I. All samples exhibit Cotton effects attributed to the phenolic chromophore because the midpoints between

(1) N. B. Eddy and E. L. May, "Synthetic Analgesics, Part IIB, 6,7-Benzomorphans," Pergamon Press, Ltd., Oxford, 1966.
(2) J. Pearl and L. S. Harris, *J. Pharmacol. Exptl. Therap.*, **154**, 319 (1966).
(3) B. F. Tullar, L. S. Harris, R. L. Perry, A. K. Pierson, A. E. Soria, W. F. Wetterau, and N. F. Albertson, *J. Med. Chem.*, **10**, 383 (1967).
(4) S. E. Fullerton, E. L. May, and E. D. Becker, *J. Org. Chem.*, **27**, 2144 (1962).
(5) W. Fedeli, G. Giacomello, S. Cerrini, and A. Vaciago, *Chem. Commun.*, 608 (1966).
(6) Y. K. Sawa and J. Irisawa, *Tetrahedron*, **21**, 1129 (1965).
(7) A. H. Beckett and P. Anderson, *J. Pharm. Pharmacol.*, **12**, 228T (1960).