six *Coleus* species other than *C. forskohlii* and the six *Plectranthus* species assayed was forskolin detected at levels as low as $\sim 1 \times 10^{-4}\%$ of the dry weight of the plant material (3).

Chromatograms from the assay of forskolin tablets are presented in Fig. 4. There was no interference due to peaks observed in the placebo samples. Assays were carried out in quadruplicate on five samples. The results indicated 5.12 mg of forskolin/tablet (label claim of 5.0 mg/tablet) with a range of 5.07–5.19 mg/tablet and a *RSD* of ±0.28%.

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Anti-Inflammatory Activity of Diazomethyl Ketone and Chloromethyl Ketone Analogs Prepared from *N*-Tosyl Amino Acids

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Abstract □ A series of diazomethyl ketone and chloromethyl ketone analogs prepared from *N*-tosyl amino acids was shown to have anti-inflammatory activity in mice at 20 mg/kg and in rats at 10 mg/kg. *N*-Tosyl-L-alanine and *N*-tosyl-β-alanine chloromethyl ketones demonstrated the most potent anti-inflammatory activity. The writhing reflex also was inhibited at 20 mg/kg in mice. In the tail flick test, *N*-tosyl-D,L-alanine and *N*-tosyl-D,L-isoleucine chloromethyl ketones demonstrated the highest increase in time. Toxicity studies indicated good therapeutic indexes for most of these agents.

Keyphrases □ Anti-inflammatory activity—diazomethyl ketone and chloromethyl ketone analogs prepared from *N*-tosyl amino acids, biological evaluation □ *N*-Tosyl amino acids—diazomethyl ketone and chloromethyl ketone analogs, evaluation for anti-inflammatory activity

A series of *N*-tosyl, *N*-benzoyl, *N*-acetyl, *N*-carbobenzyloxy, and *N*-cinnamyl cyanomethyl esters of amino acids was shown to have anti-inflammatory and immunosuppressive activities in mice (1). The standard proteolytic inhibitor tosylphenylalanyl chloromethyl ketone also was active as an anti-inflammatory agent. This report discusses a series of diazomethyl ketones and chloromethyl ketones of *N*-tosyl amino acids that demonstrated similar activity.

EXPERIMENTAL

The synthesis and physical data of this series of compounds were reported previously (2). The synthetic methods were essentially those outlined by Schoellman and Shaw (3). Male CF_1 mice (~30 g) or male Sprague–Dawley rats (~160 g) were administered the test drugs at 20 or 10 mg/kg ip, respectively, in 0.05% polysorbate 80, 3.5 hr prior to the injection of 1% carrageenan in 0.9% saline into the plantar surface of the right hindfoot. Isotonic saline was injected into the left hindfoot to obtain baseline data. After 3 hr, both feet were excised at the tibiotarsal (ankle) joint according to a modification of the method of Winter *et al.* (4, 5).

As an analgesic screen, the tail flick (6) method was employed with male CF_1 mice (~30 g) who received the test drugs at 20 mg/kg ip 5 min prior to the analgesic test. An apparatus was designed and implemented in these laboratories to measure the tail flick response time of mice (Fig. 1). A control box housed all of the timing, logic, and sensing circuitry and the feedback controls. A secondary box provided the self-contained heater (55 ± 0.5°), the temperature-sensing elements, and special custom-made optical sensors to detect the flick of the tail. The sense signals were coupled to the control box to regulate the surface temperature and to freeze the digital clock (accuracy of 0.001%) display at the time of the first tail flick.

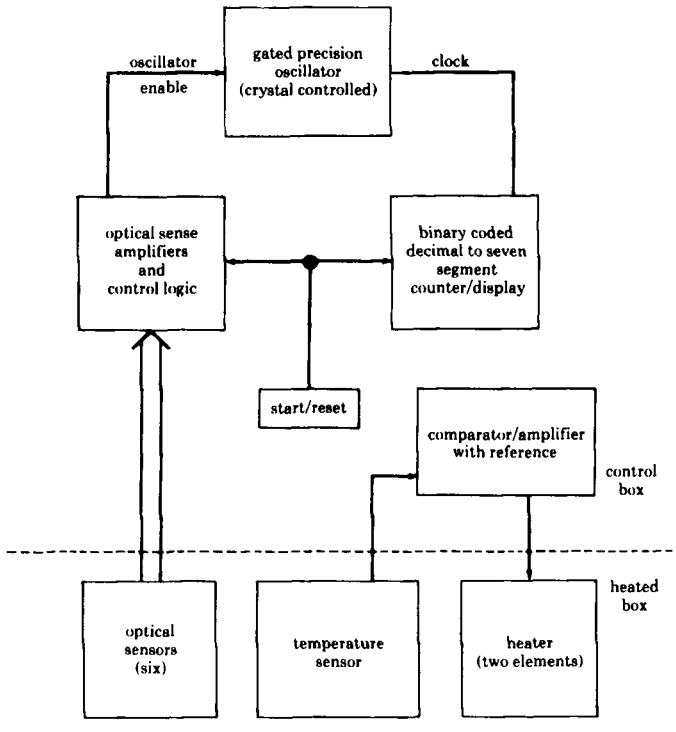

Figure 1—*Functional diagram of tail flick apparatus.*

The writhing reflex also was utilized as an analgesic test. Mice were administered the test drugs at 20 mg/kg ip 20 min prior to the administration of 0.5 ml of 0.6% acetic acid (7). After 5 min, the number of

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Table I—Anti-Inflammatory and Analgesic Activity of Diazomethyl Ketone and Chloromethyl Ketone Analogs Prepared from *N*-Tosyl Amino Acids

	Compound (n = 6)	Anti-Inflammatory Screen: Mouse at 20 mg/kg, mean[a] % inhibition ± *SD*	Anti-Inflammatory Screen: Rat at 10 mg/kg, mean % inhibition ± *SD*	Writhing Reflex at 20 mg/kg, mean % inhibition ± *SD*	Tail Flick at 20 mg/kg, mean % increase ± *SD*
I	*N*-Tosyl-L-alanine diazomethyl ketone	41 ± 2	22 ± 7	41 ± 4	168 ± 11
II	*N*-Tosyl-β-alanine diazomethyl ketone	23 ± 4	31 ± 4	43 ± 7	132 ± 9
III	*N*-Tosyl-L-valine diazomethyl ketone	25 ± 4	—	—	—
IV	*N*-Tosyl-L-leucine diazomethyl ketone	38 ± 3	48 ± 4	49 ± 7	232 ± 8
V	*N*-Tosyl-D,L-isoleucine diazomethyl ketone	34 ± 2	41 ± 9	62 ± 6	264 ± 11
VI	6-(*N*-Tosyl)aminocaproic acid diazomethyl ketone	7 ± 5[b]	—	—	—
VII	*N*-Tosyl-glycine chloromethyl ketone	29 ± 4	54 ± 4	60 ± 6	205 ± 10
VIII	*N*-Tosyl-L-alanine chloromethyl ketone	59 ± 2	61 ± 8	80 ± 3	160 ± 7
IX	*N*-Tosyl-D,L-alanine chloromethyl ketone	26 ± 4	34 ± 7	66 ± 7	419 ± 12
X	*N*-Tosyl-β-alanine chloromethyl ketone	47 ± 3	61 ± 8	62 ± 7	119 ± 10[c]
XI	*N*-Tosyl-L-valine chloromethyl ketone	39 ± 2	55 ± 6	44 ± 2	243 ± 11
XII	*N*-Tosyl-D,L-isoleucine chloromethyl ketone	45 ± 2	53 ± 7	45 ± 4	532 ± 8
XIII	*N*-Tosyl-L-proline chloromethyl ketone	53 ± 2	49 ± 7	46 ± 3	282 ± 9
XIV	6-(*N*-Tosyl)aminocaproic acid chloromethyl ketone	43 ± 2	54 ± 6	52 ± 7	215 ± 7
XV	Tosylphenylalanyl chloromethyl ketone	56 ± 2	—	—	—
XVI	Indomethacin (10 mg/kg)	74 ± 2	78 ± 8	43 ± 7	—
XVII	Phenylbutazone (50 mg/kg)	—	47 ± 5	—	—
XVIII	Morphine (1 mg/kg)	—	—	—	210 ± 7
1% Carboxymethylcellulose		0 ± 4[d]	0 ± 5[e]	0 ± 6[f]	0 ± 9[g]

[a] In the absence of a superscript, the mean inhibition is significantly higher than the control at $p \leq 0.001$. [b] Not significant. [c] $p \leq 0.010$. [d] A 59.5-mg increase in foot weight. [e] A 0.655-g increase in foot weight. [f] Seventy-eight stretch reflexes/10 min. [g] 12.21 sec for first tail flick.

stretches, characterized by repeated contractures, was counted for 10 min.

Toxicity studies were determined in male CF_1 mice (8).

RESULTS AND DISCUSSION

With the exception of VI, all of the diazomethyl ketone and chloromethyl ketone analogs of *N*-tosyl amino acids demonstrated significant anti-inflammatory activity in both mice and rats (Table I). Compounds VIII and X demonstrated the highest activity; at 20 mg/kg, they showed improved activity over phenylbutazone at 50 mg/kg in rats. In the writhing reflex, a test that mimics inflammation pain, V, VII–X, and XIV demonstrated potent activity, which was actually higher than that of indomethacin at a comparable dose. In the tail flick test, a narcotic analgesic test, the *N*-tosyl amino acid derivatives also were active and were comparable to morphine at 1 mg/kg. Although IX and XII demonstrated the best activity in the tail flick test, they did not possess the highest anti-inflammatory activity or the ability to inhibit the writhing reflex. Previous studies (2) indicated that these compounds were not toxic in mice when administered for 10 days at 20 mg/kg.

Toxicity studies in male mice indicated that I, II, IV, VI, and X–XII had an LD_{50} value of ≥400 mg/kg; VII, IX, XIII, and XIV had an LD_{50} value of >200 mg/kg. Compound V had an LD_{50} value of ≃100 mg/kg, while the value for VIII was 140 mg/kg. The solubility of the material and the limited amount of compounds curtailed further detailed studies of toxicity.

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