Escherichia coli[3] (114), *Salmonella typhi*[3] (115), *Klebsiella pneumoniae*[3] (ATCC 10031), *Pseudomonas aeruginosa*[3] (ATCC 10145), *Agrobacterium tumefaciens*[3] (NRRL B 36), *Proteus vulgaris*[4] (145), *Vibrio cholerae*[4] (ATCC 14033), *Shigella dysenteriae*[4] (ISRC 566/61), and *Mycobacterium tuberculosis*[5] ($H_{37}Rv$).

The fungi used were *Microsporum canis*[3] (VM 200-USPHS), *Microsporum gypseum*[3] (153 CSTM), *Trichophyton mentagrophytes*[3] (A 280-USPHS), *Trichophyton rubrum*[3] (252 CSTM), *Candida albicans*[3] (SKF 2270), *Cryptococcus neoformans*[3] (103), *Sporotrichum schenkii*[3] (107), *Aspergillus fumigatus*[3] (68 LI), *Histoplasma capsulatum*[3] (RNSH Hi 70½ Sydney), and *Epidermophyton floccosum*[5] (HM 300).

Antibacterial Activity—The *in vitro* antibacterial activity was determined by an agar dilution method (9). Twofold serial dilutions of the test compound were prepared in melted tryptone soya agar (oxoid), made into slopes in 18 × 150-mm test tubes. The slopes were streaked with one loopful of an overnight culture of each test organism in tryptone soya broth and incubated for 48 hr at 37°. *M. tuberculosis* was maintained on Lowenstein–Jensen medium.

Antituberculosis activity was tested in Youmans medium (10) following the serial dilution method. To 5 ml of Youmans medium containing the concentrations of the compound, one loopful (4 mm diameter) of 12–14-day-old culture was added. Cells were grown as stationary floating cultures, and growth of cells was followed visually at weekly intervals for 3 weeks.

Antifungal Activity—The compounds were tested for activity by the agar dilution assay method described by Robinson *et al.* (11). The compound under test was diluted in Sabouraud dextrose agar medium, maintained at 50°, in 18 × 150-mm test tubes and slanted. The fungi were streaked across the surface of the slants containing different concentrations of the test compound. The growth was observed visually after 3–14 days, depending upon the test organism.

In all cases, the minimum inhibitory concentration was expressed in terms of micrograms per milliliter at which the growth of the test culture was completely suppressed. A control tube containing the same medium without the test compound was included for each organism tested. Duplicates were maintained for all concentrations.

[4] Obtained from Indian Institute of Experimental Medicine, Calcutta, India.

[5] Obtained from Microbiology & Pharmacology Department, Indian Institute of Science, Bangalore, India.

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[x] To whom inquiries should be directed.

Anti-Inflammatory and Antiproteolytic Properties of 1-(1-Naphthylacetyl)-3-substituted Carbamides

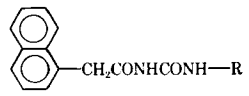
VIMAL KISHORE *[§], SUSHIL KUMAR *, SURENDRA S. PARMAR *[‡x], and VIRGIL I. STENBERG [§]

Abstract □ Several 1-(1-naphthylacetyl)-3-substituted carbamides were synthesized, characterized, and evaluated for anti-inflammatory and antiproteolytic activity. The protection afforded by most of these carbamides against carrageenan-induced edema in rats at a dose of 100 mg/kg ranged from 4.4 to 50%. Some of these carbamides, which showed higher protection against carrageenan-induced edema, were further evaluated for their antigranulation effect against cotton pellet-induced granuloma formation in rats. All carbamides showed a poor degree of protection against granuloma formation. The antiproteolytic activity of these carbamides, as reflected by their ability to inhibit trypsin-induced hydrolysis of the bovine serum albumin, was of a low order and was unrelated to their anti-inflammatory activity.

Keyphrases □ Carbamides, 1-(1-naphthylacetyl)-3-substituted—synthesized, evaluated for anti-inflammatory and antiproteolytic activity □ Anti-inflammatory activity—1-(1-naphthylacetyl)-3-substituted carbamides evaluated □ Antiproteolytic activity—1-(1-naphthylacetyl)-3-substituted carbamides evaluated □ Structure–activity relationships—1-(1-naphthylacetyl)-3-substituted carbamides synthesized and evaluated for anti-inflammatory and antiproteolytic activity

Several arylacetic acids and amides have been reported to be active anti-inflammatory agents (1–6). Earlier studies reported high anti-inflammatory activity for several derivatives of 1-naphthylacetic acid (7, 8) and substituted 1-naphthylacetamide (4, 9). Certain substituted ureas also have been reported to possess anti-inflammatory activity (10). These observations prompted the synthesis of a series of 1-(1-naphthylacetyl)-3-substituted carbamides, which were evaluated for anti-inflammatory activity against carrageenan-induced edema and cotton pellet-induced granuloma formation.

Table I—Physical Constants of 1-(1-Naphthylacetyl)-3-substituted Carbamides



Compound	R	Melting Point[a]	Yield, %	Molecular Formula[b]	Analysis, %	
					Calc.	Found
I	CH_3	197°	45	$C_{14}H_{14}N_2O_2$	C 69.42	69.46
					H 5.78	5.62
					N 11.57	11.56
II	C_2H_5	172°	56	$C_{15}H_{16}N_2O_2$	C 70.35	70.31
					H 6.25	6.24
					N 10.94	10.93
III	n-C_3H_7	141°	51	$C_{16}H_{18}N_2O_2$	C 71.11	71.35
					H 6.66	6.61
					N 10.37	10.45
IV	n-C_4H_9	120°	75	$C_{17}H_{20}N_2O_2$	C 71.83	71.69
					H 7.04	7.12
					N 9.86	9.80
V	C_6H_5	202°	70	$C_{19}H_{16}N_2O_2$	C 74.99	74.78
					H 5.26	5.12
					N 9.21	9.40
VI	o-$CH_3C_6H_4$	202°	65	$C_{20}H_{18}N_2O_2$	C 75.48	75.51
					H 5.66	5.60
					N 8.80	8.81
VII	m-$CH_3C_6H_4$	197°	58	$C_{20}H_{18}N_2O_2$	C 75.48	75.56
					H 5.66	5.68
					N 8.80	8.86
VIII	p-$CH_3C_6H_4$	188°	50	$C_{20}H_{18}N_2O_2$	C 75.48	75.48
					H 5.66	5.61
					N 8.80	8.79
IX	3,4-$(CH_3)_2C_6H_3$	190°	55	$C_{21}H_{20}N_2O_2$	C 75.91	76.14
					H 6.02	6.31
					N 8.43	8.49
X	m-$OCH_3C_6H_4$	173°	65	$C_{20}H_{18}N_2O_3$	C 71.86	71.66
					H 5.39	5.42
					N 8.38	8.45
XI	m-ClC_6H_4	185°	61	$C_{19}H_{15}ClN_2O_2$	C 67.36	67.24
					H 4.42	4.41
					N 8.26	8.28

[a] Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected. [b] All substituted carbamides, except IX, were recrystallized from ethanol; IX was recrystallized from a benzene–ethanol mixture.

Furthermore, the role of proteolytic enzymes in the inflammatory process is well documented (11, 12). The antiprotease property of various anti-inflammatory compounds has been shown to reflect their ability to inhibit enzymes causing the formation of permeability-increasing factors (13, 14). These observations prompted the investigation of the effects of substituted carbamides on protein catabolism to elucidate the biochemical basis of their anti-inflammatory property.

EXPERIMENTAL[1]

1-Naphthylacetyl Chloride—A mixture of 1-naphthylacetic acid (0.25 mole) and thionyl chloride (0.3 mole) in 100 ml of dry benzene was refluxed on a steam bath for 2–4 hr. Excess benzene and thionyl chloride were removed by distillation under reduced pressure. 1-Naphthylacetyl chloride, obtained as a residue, was distilled under reduced pressure, bp 167°/10 mm [as reported earlier (15)].

Arylcarbamides—The appropriate aromatic amine (0.1 mole) was dissolved in warm acetic acid (10 ml) containing 50 ml of water. This solution was diluted with 150 ml of water, and a warm solution of potassium cyanate (0.1 mole) in 50 ml of water was added with stirring. The arylcarbamides, which precipitated almost immediately, were allowed to stand for several hours. Then they were collected by filtration, washed with water, dried, and recrystallized from the appropriate solvents (16).

1-(1-Naphthylacetyl)-3-substituted Carbamides—A mixture of 1-naphthylacetyl chloride (0.015 mole) and the appropriate alkyl-[2] or arylcarbamide (0.015 mole) in 30 ml of dry benzene was refluxed on a steam bath. As the reaction proceeded, the alkyl- or arylcarbamide dissolved into benzene solution. Finally, when the reaction was complete, a clear solution was obtained. Excess benzene was removed by distillation under reduced pressure, and the residual mass was first extracted with a dilute solution of sodium bicarbonate to remove any acid present in the compound; this procedure was followed by thorough washing with water.

The crude products were collected by filtration, dried, and recrystallized from the appropriate solvents. The various 1-(1-naphthylacetyl)-3-substituted carbamides (Table I) were characterized by their sharp melting points, elemental analyses, and IR spectra. The presence of a characteristic band for carbonyl (1884 cm^{-1}) and amino (3185 cm^{-1}) in the IR spectra provided further support for their molecular structure. However, the substituted carbamides formed during condensation of substituted ureas with 1-naphthylacetyl chloride will possess either Structure I or II; that is, acylation could occur at the 3-*N* (I) or 1-*N* (II) position of the urea molecule.

In the present study, NMR spectra[3] of three 1-(1-naphthylacetyl)-3-substituted carbamides possessing substituents of various sizes, *i.e.*, methyl, ethyl, and phenyl, established the exact position of the substituent group and provided evidence that acylation occurs at the 3-*N*-position (I) of the urea molecule rather than the 1-*N*-position (II). The NMR spectra showed two signals for amino protons (NH^a and NH^b). Because of its direct attachment to two carbonyl groups, the signal of NH^a was assigned at a lower field than NH^b. The NMR spectra obtained after shaking these three substituted carbamides with deuterium oxide showed the absence of two signals observed for NH^a and NH^b.

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected. IR spectra were obtained with Perkin-Elmer Infracord spectrophotometer model 137, equipped with sodium chloride optics, in potassium bromide films in the 700–3500-cm^{-1} range.

[2] Aldrich Chemical Co., Milwaukee, Wis.

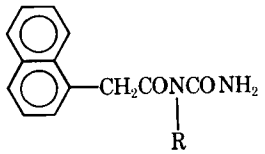
[3] Varian A-60 spectrometer.

Table II—Chemical Shifts of Some Protons of 1-(1-Naphthylacetyl)-3-substituted Carbamides

Compound	Chemical Shifts[a], ppm					
	$CDCl_3$			$CDCl_3 + D_2O$[b]		
	NH^a	NH^b	R	NH^a	NH^b	R
I ($R = CH_3$)	8.63 (1 H, broad)	8.1 (1 H, broad)	2.8 (3H, d, J = 5 Hz)	—	—	2.8 (3H, s)
II ($R = C_2H_5$)	9.58 (1H, broad)	8.41 (1H, broad)	1.12 (3H, t, J = 7Hz), 3.33 (2H, m)	—	—	1.12 (3H, t, J = 7 Hz), 3.30 (2H, q)
V ($R = C_6H_5$)	10.53 (1H, broad)	9.05 (1H, broad)	m (12H)	—	—	m (12H)

[a] Chemical shifts are expressed in δ units relative to tetramethylsilane as the internal standard. All spectra were taken in deuterochloroform solution; s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. [b] In exchange experiments with deuterium oxide, a crystal of potassium carbonate was added to the solution to promote the exchange.

Structure I



Structure II

To substantiate further the structural assignment of the methyl and ethyl carbamides, the effect of NH^b on the multiplicity of the substituent group was examined before and after deuterium exchange. As expected, the methyl signal of the carbamide changed from a doublet to a singlet; for the ethyl derivative, the methylene signal changed from a multiplet to a quartet. The chemical shifts of some protons presented in Table II clearly demonstrated that Structure I represents the substitution pattern and that acylation occurs at the 3-*N*-position rather than the 1-*N*-position of the urea molecule to form the 1-(1-naphthylacetyl)-3-substituted carbamides used in this study.

Determination of Anti-Inflammatory Activity—Albino rats, 100–120 g, were used for the evaluation of the anti-inflammatory activity of 1-(1-naphthylacetyl)-3-substituted carbamides. They were allowed food and water *ad libitum*.

Carrageenan-Induced Edema Method—The substituted carbamides, suspended in 5% aqueous gum acacia, were administered (100 mg/kg ip) to a group of six rats 1 hr before the injection of 0.05 ml of a suspension of freshly prepared carrageenan (1% in 0.91% NaCl) under the plantar aponeurosis of the right hindpaw (17). The control group of six rats received an equivalent amount of 5% aqueous gum acacia. The mean increase in paw volume, measured by the micropipet method (18) before and 4 hr after carrageenan treatment, was used to determine antiedema activity. Hydrocortisone (10 mg/kg ip) and oxyphenbutazone (40 mg/kg ip) were used as standard reference drugs.

Cotton Pellet-Induced Granuloma Formation Method—Sterilized cotton pellets (10 ± 1 mg each) were implanted into groups of four rats by making a small incision of about 2 mm on each axilla and each groin (19, 20), and the wound was stitched. One group served as the control while other groups were administered substituted carbamides (100 mg/kg ip) or oxyphenbutazone (100 mg/kg ip) every 24 hr for 6 days. On the 7th day, all implanted cotton pellets were carefully dissected out, dried for 2 hr at 150°, and weighed. The increase in weight due to granuloma formation was used to determine the anti-inflammatory activity.

Assay of Proteolytic Activity of Trypsin—The antiproteolytic activity of substituted carbamides at a final concentration of 1 m*M* was determined by evaluating their ability to inhibit trypsin-induced hydrolysis of bovine serum albumin (21). The reaction mixture consisted of 0.05 *M* tromethamine buffer (pH 8.2), 0.075 mg of crystalline trypsin (1 g is sufficient to hydrolyze 250 g of casein), 0.03 m*M* bovine serum albumin, appropriate substituted carbamide dissolved in dimethylformamide, and water in a total volume of 1 ml. An equivalent amount of dimethylformamide, added to the control tubes, had no effect on trypsin activity.

The test compounds were preincubated with trypsin for 10 min prior to the addition of bovine serum albumin, and the reaction mixture was incubated further for 5 min. The reaction was stopped by the addition of 5 ml of 15% (w/v) trichloroacetic acid solution. The

Table III—Antiedema and Antiproteolytic Activity of 1-(1-Naphthylacetyl)-3-substituted Carbamides

Compound[a]	Mean Increase in Paw Volume[b], ml ± *SE*	Antiedema Activity		Antiproteolytic Activity[c], % Inhibition
		% Protection	*p*	
Control	0.90 ± 0.02	—	—	
I	0.86 ± 0.02	4.4	>0.1	6.4 ± 0.3
II	0.60 ± 0.01	33.3	<0.001	11.0 ± 0.2
III	0.48 ± 0.03	46.6	<0.001	7.7 ± 0.2
IV	0.67 ± 0.01	30.0	<0.001	19.3 ± 0.3
V	0.45 ± 0.03	50.0	<0.001	13.5 ± 0.3
VI	0.59 ± 0.02	34.4	<0.001	4.9 ± 0.2
VII	0.69 ± 0.04	23.3	0.001	16.4 ± 0.3
VIII	0.89 ± 0.04	0.0	—	8.7 ± 0.2
IX	0.70 ± 0.02	22.2	<0.001	12.0 ± 0.2
X	0.56 ± 0.01	37.7	<0.001	14.8 ± 0.3
XI	0.91 ± 0.02	0.0	—	3.9 ± 0.2
Hydrocortisone	0.47 ± 0.02	47.7	<0.001	—
Oxyphenbutazone	0.45 ± 0.03	50.0	<0.001	—
Sodium salicylate	—	—	—	55.4 ± 0.3

[a] Substituent groups are as indicated in Table I. [b] Assay procedures are as indicated in the text. All substituted carbamides were used in a dose of 100 mg/kg, while hydrocortisone and oxyphenbutazone were used in a dose of 10 and 40 mg/kg, respectively. [c] Assay procedures are as indicated in the text. Each experiment was done in duplicate and the values are the mean values with ± *SEM* calculated from two separate experiments. All substituted carbamides and sodium salicylate were used at a final concentration of 1 m*M*.

Table IV—Antigranulation Effect of 1-(1-Naphthylacetyl)-3-substituted Carbamides

Compound[a]	Average Weight of Granulation Tissue, mg ± SE	Antigranulation Activity	
		% Protection	p
Control	17.6 ± 0.49	—	—
II	15.8 ± 0.44	10.2	0.01
III	16.6 ± 0.82	5.6	>0.1
IV	15.6 ± 0.61	11.3	0.02
V	14.8 ± 0.77	15.9	0.001–0.01
VI	16.9 ± 0.81	3.9	>0.1
X	16.2 ± 0.55	7.9	0.05
Oxyphenbutazone	9.0 ± 0.36	48.8	<0.001

[a] Substituent groups are as indicated in Table I. Assay procedures are as indicated in the text. All substituted carbamides and oxyphenbutazone were used in a dose of 100 mg/kg.

acid-soluble products of protein breakdown, obtained after centrifugation, were determined (22) as an index of the enzyme activity. Sodium salicylate also was used at a final concentration of 1 m*M* for comparative evaluation of antiproteolytic activity.

RESULTS AND DISCUSSION

The anti-inflammatory activity of these substituted carbamides against carrageenan-induced edema is recorded in Table III. Most of these carbamides afforded protection ranging from 4.5 to 50%. However, 1-(1-naphthylacetyl)-3-*p*-tolylcarbamide (VIII) and 3-*m*-chlorophenylcarbamide (XI) were devoid of anti-inflammatory activity. Hydrocortisone and oxyphenbutazone, under similar experimental conditions, provided protection of 47 and 50% in doses of 10 and 40 mg/kg, respectively, against carrageenan-induced edema as compared to substituted carbamides used in a dose of 100 mg/kg.

As is evident from Table III, an increase in the number of the carbon atoms in the alkyl chain attached to the nitrogen atom of these carbamides influenced their ability to protect against carrageenan-induced edema. The degree of protection was in the order of methyl < ethyl < *n*-propyl > *n*-butyl, and maximum anti-inflammatory activity was observed with the *n*-propyl-substituted carbamide (III).

Attachment of a methyl or methoxy substituent on the phenyl nucleus attached to the nitrogen atom of these aryl-substituted carbamides decreased their antiedema effectiveness. The introduction of a *p*-methyl or *m*-chloro substituent on the phenyl nucleus completely abolished the anti-inflammatory activity of the phenyl-substituted carbamide (V).

All substituted carbamides possessed low antiproteolytic activity, as reflected by only 3.9–19.3% inhibition of trypsin activity during hydrolysis of bovine serum albumin (Table III). Such an inhibition of trypsin-induced hydrolysis of bovine serum albumin was significantly higher (55.4%) with sodium salicylate, used as a reference drug for comparative evaluation. These results failed to provide any correlation between antiedema activity of these substituted carbamides and their ability to inhibit trypsin-induced hydrolysis of bovine serum albumin.

Some of the substituted carbamides possessing higher antiedema activity were evaluated also for their antigranulation activity. Their ability to protect against cotton pellet-induced granuloma formation in rats was of a low order (Table IV). The decrease in the weight of granuloma formation in implanted cotton pellets was 4–16% as compared to oxyphenbutazone, which showed 48.8% antigranulation activity at 100 mg/kg, a dose shown to exhibit 50% (ED_{50}) protection against cotton pellet-induced granuloma formation (23). In the present study, as in the case of antiedema activity, 1-(1-naphthylacetyl)-3-phenylcarbamide (V) exhibited maximum antigranulation activity of 15.9% against cotton pellet-induced granuloma formation.

These results indicated that 1-(1-naphthylacetyl)-3-substituted carbamides are active only against the acute phase of inflammation and provide poor protection against the chronic phase of inflammatory reactions. Failure to observe a relationship between antiedema and antigranulation activity of these substituted carbamides indicated that different mechanisms presumably are responsible for the acute and the chronic inflammatory reactions. In addition, the low antiproteolytic activity observed in the present study cannot account for the biochemical basis for the antiedema activity of these 1-(1-naphthylacetyl)-3-substituted carbamides.

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