

1.06 (3 H, s), 5.39 (1 H, d, $J = 10$ Hz), 5.89 (1 H, dd, $J = 10$ and 2 Hz), 6.06 (1 H, s). Anal. ($C_{24}H_{26}O_3$) C, H.

3-(17 β -Hydroxy-6 β ,7 β :15 β ,16 β -bis(methylene)-3-oxo-androsta-1,4,11-trien-17 α -yl)propionic Acid γ -Lactone (45). A 201-mg (0.55 mmol) sample of 44 was oxidized with 185 mg (1.6 mmol) of SeO_2 and 0.03 mL of pyridine in 20 mL of *tert*-butyl alcohol. The product was purified by the procedure described for the preparation of 14a, giving 116 mg (58.2%) of 45 as a

noncrystalline powder: IR ($CHCl_3$) 1594, 1619, 1660, 1767 cm^{-1} ; NMR ($CDCl_3$) δ 1.08 (6 H, s), 5.52 (1 H, d, $J = 10$ Hz), 5.92 (1 H, dd, $J = 10$ and 2 Hz), 6.18 (1 H, dd, $J = 10$ and 2 Hz), 6.34 (1 H, d, $J = 2$ Hz), 6.97 (1 H, d, $J = 10$ Hz).

Acknowledgment. We express our sincere thanks to Dr. Ken'ichi Takeda and Dr. Wataru Nagata for their interest in this work and helpful discussions.

Analgesic Dipeptide Derivatives. 3. Synthesis and Structure-Activity Relationships of *o*-Nitrophenyl-Modified Analogues of the Analgesic Compound H-Lys-Trp(NPS)-OMe¹

M. Teresa Garcia-López,*† Rosario González-Muñiz,† M. Teresa Molinero,‡ José R. Naranjo,‡ and J. Del Rio‡

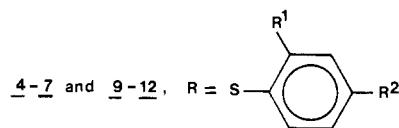
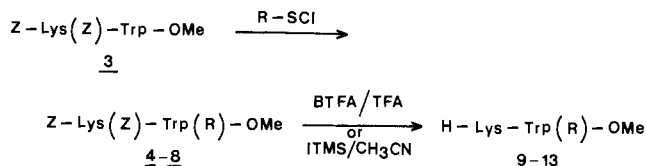
Instituto de Química Médica, C.S.I.C., Juan de la Cierva 3, 28006 Madrid, Spain, and Instituto Cajal, C.S.I.C., Velázquez 144, 28006 Madrid, Spain. Received February 2, 1987

A series of analogues of the analgesic dipeptide derivative H-Lys-Trp(NPS)-OMe has been designed to determine the influence of the (2-nitrophenyl)sulfonyl (NPS) moiety on the activity. The syntheses and antinociceptive effects of these analogues of general formula H-Lys-Trp(R)-OMe [R = phenylsulfonyl (PS) (9); R = (2-carbomethoxyphenyl)sulfonyl (CmPS) (10); R = (4-nitrophenyl)sulfonyl (*p*NPS) (11); R = (2,4-dinitrophenyl)sulfonyl (DNPS) (12); R = [2-(acetylamino)-2-carbomethoxyethyl]sulfonyl (AacCmES) (13); R = [2-(acetylamino)phenyl]sulfonyl (AacPS) (17); R = *tert*-butylsulfonyl (*t*-BuS) (23); R = (2-carbomethoxyethyl)sulfonyl (CmES) (24)] are described. Reaction of Z-Lys(Z)-Trp-OMe (3) with PS-, CmPS-, *p*NPS-, DNPS-, and AacCmES-Cl afforded the corresponding 2-(sulfonyl)tryptophan derivatives, which on treatment with boron-tris(trifluoroacetate)/trifluoroacetic acid or trimethylsilyl iodide in acetonitrile (Me_3SiI/CH_3CN) provided 9-13, respectively. Sulfonylation of 3 with NPS-Cl gave Z-Lys(Z)-Trp(NPS)-OMe, which, on catalytic hydrogenation of the nitro group using 10% Pd/C followed by acetylation of the resulting amino function and removal of the protecting Z groups, gave 17. Condensation of 2-(*tert*-butylsulfonyl)- and 2-[(2-carbomethoxyethyl)sulfonyl]tryptophan methyl ester, obtained by reaction of methyl 3 α -hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylate with the corresponding thiol, with Z-Lys(Z)-OSu afforded Z-Lys(Z)-Trp(*t*-BuS)-OMe and Z-Lys(Z)-Trp(CmES)-OMe, which on treatment with Me_3SiI/CH_3CN provided 23 and 24, respectively. Intracerebroventricular administration of 10 elicited a naloxone-reversible antinociceptive effect in mice similar to that of H-Lys-Trp(NPS)-OMe. No analgesia was however found with the phenylsulfonyl or acyclic sulfonyl substituted dipeptides 9, 11, and 17 or 13, 23, and 24. The Trp(DNPS)-containing analogue was neurotoxic. Structure-activity studies indicate that the role of the NPS and CmPS moieties could be related to the adoption of a preferential active conformation.

In the first paper of this series,² we have shown that the synthetic dipeptide derivative H-Lys-Trp(NPS)-OH [NPS = (*o*-nitrophenyl)sulfonyl] (1) exhibited a naloxone-reversible analgesia in mice, when administered intracerebroventricularly, comparable with that of the enkephalin analogue D-Ala²-Met-enkephalinamide (DAME) regarding both the maximum effect and the time-course of analgesia. A similar antinociceptive effect has also been found with the corresponding dipeptide methyl ester, H-Lys-Trp(NPS)-OMe (2). However, no analgesia was observed with Trp(NPS) or with the unsubstituted dipeptide H-Lys-Trp-OH. Preliminary studies to establish the structural requirements for the antinociceptive effect of 1 and 2 revealed, besides the importance of the NPS moiety, the need for a basic amino acid, although the side-chain length was not a critical factor.^{2,3} In view of the peculiar structure of 1 and 2 as compared to other directly or indirectly acting opioid peptides, we have considered it of interest to gain further insight into the structure-activity relationships. In this sense, the role of the NPS moiety seemed to us rather intriguing, and, therefore, we have now investigated the effect of replacing this portion by various related groups.

The present paper describes the synthesis and analgesic activity in mice of a series of novel H-Lys-Trp(R)-OMe in which the substituent R at the 2-position of Trp is a

Scheme I



$\underline{4}$ and $\underline{9}$; $R^1 = R^2 = H$ (PS)

$\underline{5}$ and $\underline{10}$; $R^1 = CO_2Me$; $R^2 = H$ (CmPS)

$\underline{6}$ and $\underline{11}$; $R^1 = H$; $R^2 = NO_2$ (*p*NPS)

$\underline{7}$ and $\underline{12}$; $R^1 = R^2 = NO_2$ (DNPS)

$\underline{8}$ and $\underline{13}$, $R = S-CH_2-CH(NHAc)-CO_2Me$ (AacCmES)

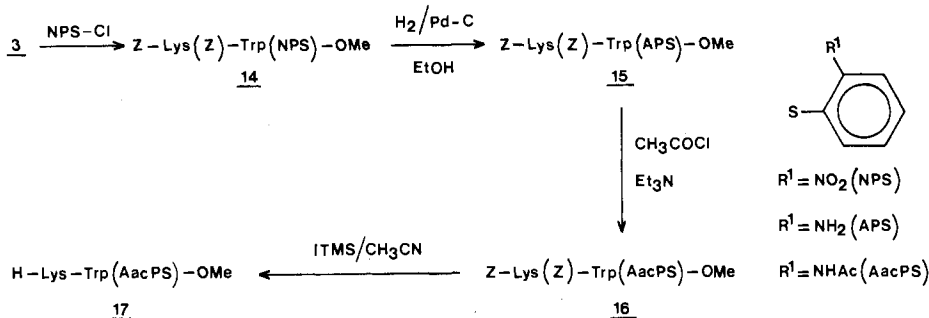
phenylsulfonyl or an acyclic sulfonyl moiety. In the first case, the *o*-nitrophenyl portion of 2 has been modified by

*Instituto de Química Médica.

†Instituto Cajal.

(1) For previous papers in this series see ref 2 and 3.

Scheme II

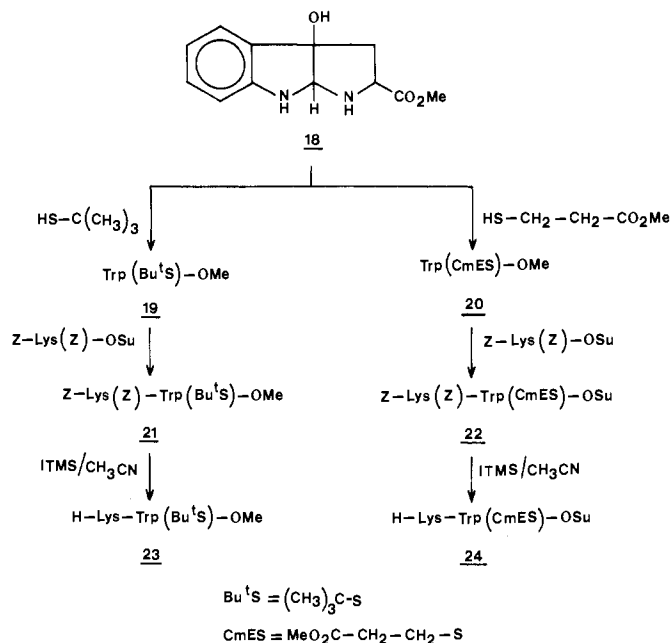


changing either the NO₂ group or its position.

Chemistry

It is known that sulfonyl halides react with Trp-containing peptides in acidic media to give the 2-thioether derivatives.⁴ On this basis, reaction of Z-Lys(Z)-Trp-OMe (3)⁵ with phenylsulfonyl chloride (PS-Cl), (2-carbomethoxyphenyl)sulfonyl chloride (CmPS-Cl),⁶ (4-nitrophenyl)sulfonyl chloride (*p*NPS-Cl), and (2,4-dinitrophenyl)sulfonyl chloride (DNPS-Cl) in dry 1 N HCl in dioxane afforded the corresponding 2-sulfonyltryptophan derivatives, Z-Lys(Z)-Trp(R)-OMe 4–7 (4, R = PS; 5, R = CmPS; 6, R = *p*NPS; 7, R = DNPS) (Scheme I). The sulfonylating agents PS- and *p*NPS-Cl were prepared according to the literature procedures,^{7,8} while the unknown CmPS-Cl was obtained by chlorinolysis in dichloromethane of 2,2'-dicarbomethoxydiphenyl disulfide⁹ using fuming H₂SO₄ as catalyst. [2-(Acetylamino)-2-carbomethoxyethyl]sulfonyl chloride (AacCmES-Cl)⁶ was generated by treatment of Ac-Cys-OMe with sulfonyl chloride in methylene chloride.¹⁰ This sulfonylating agent in situ reacted with the protected dipeptide 3 to give the corresponding Trp(AacCmES) derivative 8. Although all these sulfonylation reactions could be achieved by using the unprotected dipeptide, we have preferred to start from the di-Z-protected derivative 3 in order to facilitate the subsequent chromatographic purifications. Removal of the Z groups

Scheme III



of compounds 4–8 utilizing boron-tris(trifluoroacetate)/trifluoroacetic acid (BTFA/TFA)¹¹ for 4–6 and trimethylsilyl iodide in acetonitrile (Me₃SiI/CH₃CN)¹² for 7 and 8 provided the corresponding deprotected analogues H-Lys-Trp(R)-OMe 9–13.

A similar sulfonylation reaction of the protected dipeptide 3, using *o*-nitrophenyl)sulfonyl chloride (NPS-Cl), gave the NPS derivative 14, which, upon hydrogenolysis of the NO₂ group at room temperature and 45 psi employing 10% Pd/C as catalyst, readily provided the corresponding (aminophenyl)sulfonyl (APS) analogue Z-Lys(Z)-Trp(APS)-OMe, 15 (Scheme II). Although simultaneous removal of the Z groups could be expected under these conditions, the ¹H NMR spectrum of compound 15 clearly demonstrated the presence of both Z groups. The fact that the Z groups remain under these conditions has been previously observed in the hydrogenolysis of a Z-Ala(N₃)-containing dipeptide,³ and it could be attributed to the catalyst poisoning caused by the resulting amine derivatives, which are rapidly formed by reduction of the nitro or azido groups.¹³ Pure samples of the Trp(APS)-containing dipeptide 15 were obtained when recently prepared; however, analytical TLC demonstrated that this compound decomposed on standing. This result

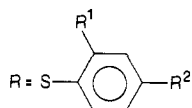
- (2) Garcia-López, M. T.; Herranz, R.; González-Muñiz, R.; Naranjo, J. R.; De Ceballos, M. L.; Del Rio, J. *Peptides* 1986, 7, 39.
- (3) Garcia-López, M. T.; González-Muñiz, R.; Herranz, R.; Molinero, M. T.; Del Rio, J. *Int. J. Peptide Protein Res.* 1987, 29, 613.
- (4) Scoffone, E.; Fontana, A.; Rocchi, R. *Biochemistry* 1968, 7, 971.
- (5) Szekerke, M.; Erchengyi, J.; Sági, J. *Acta Chim. Acad. Sci. Hung.* 1974, 80, 183.
- (6) Unusual abbreviations (CmPS, AacCmES, AacPS, CmES) have been chosen to make them understandable and in agreement with abbreviations that are in general use, such as PS, NPS, *p*NPS, and DNPS. Small letters indicate those functions that are attached to the group indicated in the preceding capital letter. (Carbomethoxyphenyl)sulfonyl has been used, instead of [(methoxycarbonyl)phenyl]sulfonyl, with the end of abbreviating this group as CmPS, since CPS has been previously used to represent the free acid [(carboxyphenyl)sulfonyl group] (Parikh, I.; Omenn, G. S. *Biochemistry* 1971, 10, 1173). Accordingly, CmES has been employed to represent (carbomethoxyethyl)sulfonyl. In order to employ homogeneous abbreviations and nomenclature, the MeO₂CCH₂(NHAc)CH₂S moiety has not been named as a cysteine derivative. For the same reason, compounds 8 and 13 have not been named as tryptathionine-containing dipeptides.
- (7) Mueller, W. H.; Butter, P. E. *J. Am. Chem. Soc.* 1968, 90, 2075.
- (8) Raban, M.; Jones, F. B., Jr. *J. Am. Chem. Soc.* 1971, 93, 2692.
- (9) Urbanski, T.; Falicki, J.; Halski, L. *Rocz. Chem.* 1956, 30, 969; *Chem. Abstr.* 1957, 51, 14595.
- (10) Plate, R.; Nivard, R. J. F.; Ottenheijm, H. C. J. *Tetrahedron* 1986, 42, 4503.

- (11) Pless, J.; Bauer, W. *Angew. Chem., Int. Ed. Engl.* 1973, 12, 147.
- (12) Lott, R. S.; Virander, S. C.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* 1979, 495.
- (13) House, H. O. *Modern Synthetic Reactions*; W. A. Benjamin: New York, 1972; Chapter 1, pp 9–18.

Table I. Physical Properties and UV Data of H-Lys-Trp(R)-OMe

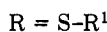
no.	R	yield, ^a %	mp, ^b °C	formula ^c	scheme	UV λ _{max} (EtOH), nm (ε)
9	PS	78	218–219	C ₂₄ H ₃₀ N ₄ O ₃ S	I	286 (18 800)
10	CmPS	99	112–113	C ₂₆ H ₃₂ N ₄ O ₃ S	I	290 (7800)
11	pNPS	74	82–84	C ₂₄ H ₂₉ N ₅ O ₃ S	I	292 (10 800), 320 (8450)
12	DNPS	92	foam	C ₂₄ H ₂₉ N ₆ O ₃ S	I	275 (17 200), 290 (13 400)
13	AacCmES	84	200 dec	C ₂₄ H ₃₅ N ₅ O ₆ S	I	290 (3950)
17	AacPS	84	198 dec	C ₂₆ H ₃₃ N ₅ O ₄ S	II	284 (3800)
23	<i>t</i> -BuS	81	foam	C ₂₂ H ₃₄ N ₄ O ₃ S·H ₂ O	III	285 (8100), 290 (8350)
24	CmES	85	foam	C ₂₂ H ₃₂ N ₄ O ₃ S	III	289 (4500)

^a Yield from the corresponding di-Z-protected derivative. ^b Crystallized from *i*-PrOH/ether. ^c Analytical results for C, H, N, and S were within ±0.4% of the theoretical values.

Table II. Partial Proton Chemical Shifts (ppm from Me₄Si) of H-Lys-Trp(R)-OMe in Me₂SO-*d*₆ at 300 MHz

no.	R ¹	R ²	phenylsulfenyl protons					Trp			Lys	
			H-2	H-3	H-4	H-5	H-6 ^c	H-α	H'-β	H''-β	H-α	H-ε
2	NO ₂	H		8.32	7.44	7.60	6.70 (7.58)	4.60	3.30	3.24	3.67	2.74
9	H	H	7.19	7.02	7.12	7.02	7.20 (7.32)	4.61	3.44	3.29	3.58	2.74
10	CO ₂ Me	H		7.99	7.25	7.40	6.54 (7.43)	4.56	3.26	3.20	3.72	2.73
11	H	NO ₂	7.20	8.14		8.14	7.20 (7.58)	4.58	3.30	3.24	3.72	2.73
12	NO ₂	NO ₂		8.91		8.33	6.92 (7.84)	4.63	3.32	3.14	<i>b</i>	2.75
17	NHAc	H		7.28	7.05	7.16	6.70 (7.25)	4.55	3.32	3.25	3.79	2.75

^a Values in parentheses are theoretical chemical shifts, calculated for the corresponding substituted phenyl-phenylthio derivative using electronic parameters. ^b Included with the H₂O signal contained in Me₂SO-*d*₆.

Table III. Partial Proton Chemical Shifts (ppm from Me₄Si) of H-Lys-Trp(R)-OMe in Me₂SO-*d*₆ at 300 MHz

no.	R ¹	Trp			Lys		OMe	R ¹ protons
		H-α	H'-β	H''-β	H-α	H-ε		
13	CH ₂ CH(NHAc)CO ₂ Me	4.58	3.30	3.14	3.72	2.80	3.54	4.28 (CH ₂); 3.60 (CO ₂ Me); 3.23 (CH); 1.90 (NAc)
23	C(CH ₃) ₃	4.53	3.32	3.23	3.78	2.76	3.40	1.24 [(CH ₃) ₃]
24	(CH ₂) ₂ CO ₂ Me	4.53	3.27	3.19	3.67	2.77	3.46	3.53 (CO ₂ Me); 3.04 (SCH ₂); 2.50 (CH ₂ CO ₂ Me)

led us to acetylate the aromatic amino group and to use the corresponding derivative for our purposes. Reaction of **15** with acetyl chloride in dichloromethane, in the presence of triethylamine, followed by treatment of the resulting [(acetylamino)phenyl]sulfenyl (AacPS)⁶ derivative **16** with Me₃SiI/CH₃CN, provided H-Lys-Trp-(AacPS)-OMe (**17**).

Among the dipeptide derivatives bearing an acyclic sulfenyl moiety at the 2-position of Trp, we considered it of interest to obtain those bearing the *tert*-butylsulfenyl (*t*-BuS) and (2-carbomethoxyethyl)sulfenyl (CmES)⁶ groups. The former is a very bulky group, and the latter could be thought of as a flexible analogue of the CmPS moiety in that both of them have a CO₂Me substituent linked to the sulfur atom by a two-carbon bridge. Since, on one hand, the aliphatic sulfenyl chlorides are much less stable than the aromatic sulfenyl chlorides and, on the other, chlorination of *tert*-alkyl disulfides cleaves the S-C bond rather than the S-S bond,¹⁴ we discarded the use of *t*-BuS- and CmES-Cl in the direct sulfenylation of the Trp moiety. Another synthetic route to the synthesis of sulfide derivatives at the 2-position of the indole ring of tryptophan is that involving the reactions of 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid with aliphatic thiols.¹⁵ Following this route, we have prepared the *t*-BuS and CmES⁶ derivatives of tryptophan

19 and **20**, by reaction in TFA of the corresponding thiols with the hexahydropyrrolo[2,3-*b*]indole methyl ester **18**,¹⁶ obtained by peroxyacetic acid oxidation of Trp-OMe.¹⁷ Condensation of the succinimido ester of Z-Lys(Z)¹⁸ with **19** and **20** in dry THF afforded the corresponding protected dipeptide derivatives **21** and **22**, which on treatment with Me₃SiI/CH₃CN, as deblocking agent for the cleavage of the Z groups, gave the desired Trp(*t*-BuS)- and Trp-(CmES)-containing dipeptides **23** and **24**, respectively (Scheme III).

The structures of all these sulfenyl-substituted dipeptides were confirmed by analytical and spectroscopic methods (Tables I–III). The UV spectrum of **9** showed an absorption maximum at 286 nm, while compound **11** absorbed at 320 nm (Table I). Both wavelength values are in good agreement with those reported in the literature for the characteristic absorptions of Trp(PS)- and Trp-(pNPS)-containing peptides.⁴ Evidence for the presence of the PS, CmPS, pNPS, DNPS, AacCmES, AacPS, *t*-BuS, and CmES substituents in compounds **9**–**12**, **13**, **17**, **23**, and **24**, respectively, came from their ¹H NMR spectra, which indicated the absence of the indole H-2 proton and the presence of signals attributable to these groups (Tables II and III). At this point, it is interesting to note that a

(14) Behforouz, M.; Kerwood, J. E. *J. Org. Chem.* **1969**, *34*, 51.

(15) Savige, W. E.; Fontana, A. *J. Chem. Soc., Chem. Commun.* **1976**, 600.

(16) Nakagawa, M.; Kaneko, T.; Yoshikawa, K.; Hino, T. *J. Am. Chem. Soc.* **1974**, *96*, 624.

(17) Savige, W. E. *Aust. J. Chem.* **1975**, *28*, 2275.

(18) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839.

significant shielding of the phenyl H-6 proton of the CmPS and DNPS derivatives 10 and 12 (δ 6.54 and 6.92, respectively) is observed when these δ values are compared with those theoretically calculated by using only electronic parameters (Table II). This shielding is identical with that previously observed in all the Trp(NPS)-containing dipeptides,³ as it is shown for compound 2, which has been included in this table for comparison. However, a good agreement between the experimental and theoretical δ values for this proton is obtained for the PS-substituted dipeptide 9. In the case of the *p*NPS derivative 11, the phenyl H-6 proton appeared at δ 7.20, although a value identical with that of the NPS analogue 2 (δ 6.70) would be expected. This significant difference ($\Delta\delta = 0.5$ ppm), which indicates a different orientation of this proton between both compounds, must be caused by a considerable distortion of the Trp(NPS) moiety of 2, with respect to the less sterically hindered Trp(*p*NPS) group of 11, to accommodate the bulky NPS substituent. This distortion of the Trp(NPS) derivatives must be such that the phenyl group is not in the plane of the indole ring and, therefore, the phenyl H-6 proton is subject to a notable shielding because of the indole ring current effect. Evidently, this explanation can be extended to the CmPS and DNPS derivatives 10 and 12. Examination of molecular models of compounds 2, 10, and 12 reveals that the steric hindrance, caused by the bulky ortho-substituted phenyl moieties, forces the *o*-NO₂ and *o*-CO₂Me substituents to be directed towards the interior of the peptide molecule and the H-6 phenyl proton to lie close above or below the plane of the indole ring. This fact supports the explanation mentioned above.

Biological Results: Structure-Activity Relationships

The antinociceptive activity in mice of the H-Lys-Trp-OMe derivatives 9-13, 17, 23, and 24 given by the icv route is listed in Table IV. For comparative purposes H-Lys-Trp(NPS)-OMe (2) and the enkephalin analogue DAME have been included. As shown in this table, the Trp-(CmPS) derivative 10 exhibited an analgesic effect comparable to that of compound 2 and slightly lower than that of the enkephalin analogue DAME. In all cases, the analgesia was almost completely blocked by previous administration of naloxone, 1 mg/kg sc, given 15 min before the icv injection. Although this antagonism suggests an opioid involvement in the antinociceptive effect of both compounds, they did not displace the binding of [³H]naloxone to mouse-brain homogenates at a concentration of 10⁻⁴ M. As in the case of the previously studied analogue H-Lys-Trp(NPS)-OH (1), it may be expected that these dipeptide derivatives do not act directly on opioid receptors and antinociception could possibly be explained by the peptidase-inhibiting and enkephalin-releasing properties of the new compounds.² No analgesia was however found with the unsubstituted phenyl analogue 9 or when the NO₂ substituent of 2 was replaced by the NHAc group (see 17). The presence of an additional NO₂ group in the DNPS derivative 12 resulted in a high neurotoxicity (see Table IV). At first sight, these findings could suggest the need for an electron-withdrawing substituent on the phenyl ring; however, the lack of activity of the *p*NPS analogue 11 discards this suggestion. Therefore, although the influence of electronic factors caused by the NO₂ and CO₂Me substituents on the antinociceptive effects of 2 and 10 cannot be excluded, this possible influence should not be the only factor involved in the analgesic effect. An explanation based exclusively on steric factors does not seem plausible either, since compound 23, bearing a bulky

Table IV Analgesic Response to the Dipeptide Derivatives 2, 9-13, 17, 23, and 24 in the Tail-Flick Test in Mice

compd	dose, μg/mouse icv	% change in reaction time (min) ^a		
		5	30	60
saline		6 ± 7	-2 ± 8	-5 ± 7
DAME	0.1	40 ± 8*	26 ± 10	-7 ± 5
	0.5	122 ± 12*	76 ± 6*	35 ± 6*
	1	210 ± 26*	118 ± 13*	45 ± 12*
2	0.1	23 ± 4	15 ± 3	7 ± 5
	0.5	98 ± 21*	50 ± 18*	20 ± 7
	1	140 ± 23*	73 ± 11*	45 ± 6*
9	0.5	14 ± 7	5 ± 3	9 ± 7
	1	12 ± 6	-2 ± 9	-5 ± 4
	2.5	15 ± 4	12 ± 5	1 ± 7
10	0.1	23 ± 10	19 ± 9	17 ± 5
	0.5	91 ± 16*	41 ± 11*	20 ± 12
	1	130 ± 28*	63 ± 19*	33 ± 7*
11	0.5	29 ± 10	23 ± 9	3 ± 5
	1	30 ± 7	31 ± 7	25 ± 8
	2.5	<i>b</i>	<i>b</i>	<i>b</i>
12	0.1	21 ± 16	23 ± 15	28 ± 12
	0.5	<i>b</i>	<i>b</i>	<i>b</i>
13	0.5	-3 ± 7	-9 ± 12	0 ± 9
	1	0 ± 8	7 ± 3	-5 ± 8
	2.5	10 ± 11	-2 ± 9	3 ± 6
17	0.5	0 ± 7	-2 ± 7	5 ± 4
	1	25 ± 14	20 ± 11	15 ± 12
	2.5	27 ± 10	26 ± 9	10 ± 8
23	0.5	0 ± 2	-1 ± 7	-1 ± 4
	1	5 ± 9	7 ± 4	9 ± 11
	2.5	-10 ± 11	5 ± 4	9 ± 3
24	0.5	11 ± 5	10 ± 5	9 ± 5
	1	15 ± 10	7 ± 8	9 ± 4
	2.5	16 ± 15	15 ± 11	0 ± 6

^aResults are the means ± SE obtained with groups of 10-12 mice. (*) Significant change ($p < 0.05$ or better, Student's *t* test).

^bSigns neurotoxicity consisting of motor incoordination, signs of respiratory disturbances, and barrel rotations.

t-BuS group, did not show an analgesic effect. As the active compound 10, the Trp(AacCmES) and Trp(CmES) derivatives 13 and 24 have a CO₂Me substituent linked to the sulfur atom by a two-carbon bridge; however, these acyclic sulfenyl derivatives were inactive. This fact could be related to the marked difference in conformational constraint between the 2-substituted phenylsulfenyl derivative 10 and the 2-substituted ethylsulfenyl analogue 13 and 24. This suggestion would fit in a general explanation based on conformational aspects, which seems to be the most likely to account for the activity of the rather rigid Trp(NPS) and Trp(CmPS) derivatives 2 and 10, the only active compounds in this series. According to the characteristic features observed in the ¹H NMR spectra of both derivatives, the active conformation appears to be one in which the phenyl and indole rings are not coplanar. Therefore, the NPS and CmPS moieties of 2 and 10 seem to play an important role in the adoption of the biologically active conformation. Nevertheless, whether the role of these moieties is exclusively related with conformational aspects or there are other additional factors, such as some type of interaction between the NO₂ or CO₂Me groups and other parts of the molecule, must be clarified. With this aim, new modifications of these Trp(NPS)- and Trp-(CmPS)-containing dipeptides and a farther conformational study using computer models are in progress.

Chemical Methods

Melting points were measured with a Kofler hot-stage apparatus and are uncorrected. ¹H NMR spectra were recorded with a Varian EM-390 or a Varian XL-300 spectrometer operating at 90 or 300 MHz, respectively, using Me₄Si as internal standard and CDCl₃ or Me₂SO-*d*₆ as sample solvents. UV absorption spectra were taken with a Perkin-Elmer 550 SE spectrophotometer

using EtOH as sample solvent. Analytical TLC was performed on aluminum sheets coated with a 0.2-mm layer of silica gel 60F₂₅₄ (Merck). Preparative layer chromatography was performed on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF₂₅₄ (Merck). Silica gel 60 (230–400 mesh) (Merck) was used for column chromatography. Compounds were detected with UV light (254 nm). (*o*-Nitrophenyl)sulfonyl chloride (NPS-Cl) and (2,4-dinitrophenyl)sulfonyl chloride (DNPS-Cl) were from Sigma. Phenylsulfonyl chloride (PS-Cl), (4-nitrophenyl)sulfonyl chloride (*p*NPS-Cl), and [2-(acetyl-amino)-2-carbomethoxyethyl]sulfonyl chloride (AacCmES-Cl) were prepared according to the literature procedures.^{7,8,10}

(2-Carbomethoxyphenyl)sulfonyl Chloride (CmPS-Cl). A suspension of methyl 2,2'-dicarbomethoxydiphenyl disulfide¹⁰ (0.4 g, 1.2 mmol) in 1,2-dichloroethane (20 mL) containing 1 drop of fuming H₂SO₄ was stirred at room temperature while Cl₂ was bubbled into the suspension. The resulting clear solution was evaporated to dryness to give a gummy residue, which was immediately used without further purification.

Phenylsulfonylation of Z-Lys(Z)-Trp-OMe (3). General Procedure. The sulfonyl chloride derivative (1.5 mmol) was added to a solution of the dipeptide derivative 3⁵ (1 mmol) in 1 N HCl/dioxane (10 mL), and the mixture was stirred at room temperature for 30 min. Removal of the solvent left a residue, which was purified by preparative TLC with EtOAc/hexane (2:1) and recrystallized from EtOAc/hexane.

Z-Lys(Z)-Trp(PS)-OMe (4): yield 86%; mp 63–64 °C; ¹H NMR (CDCl₃, 90 MHz) δ 8.60 (brs, 1 H, NH^{im}), 7.60–6.90 (m, 5 H, PS 2, 3, 4, 5, 6), 7.30 (s, 10 H, Z C₆H₅), 5.00 (s, 4 H, Z CH₂), 4.86 (m, 1 H, Trp α CH), 4.00 (m, 1 H, Lys α CH), 3.60 (s, 3 H, OMe), 3.32 (m, 2 H, Trp β CH₂), 3.03 (m, 2 H, Lys ε CH₂). Anal. (C₄₀H₄₂N₄O₇S) C, H, N, S.

Z-Lys(Z)-Trp(CmPS)-OMe (5): yield 86%; mp 73–74 °C; ¹H NMR (CDCl₃, 90 MHz) δ 8.75 (brs, 1 H, NH^{im}), 7.95 (dd, 1 H, CmPS 3), 7.34 (s, 10 H, Z C₆H₅), 6.60 (dd, 1 H, CmPS 6), 5.00 (s, 4 H, Z CH₂), 4.87 (m, 1 H, Trp α CH), 4.05 (m, 1 H, Lys α CH), 3.87 (s, 3 H, CmPS CO₂CH₃), 3.61 (s, 3 H, OMe), 3.30 (m, 2 H, Trp β CH₂), 3.05 (m, 2 H, Lys ε CH₂). Anal. (C₄₂H₄₄N₄O₉S) C, H, N, S.

Z-Lys(Z)-Trp(*p*NPS)-OMe (6): yield 94%; mp 70–72 °C; ¹H NMR (CDCl₃, 90 MHz) δ 9.00 (brs, 1 H, NH^{im}), 7.93 (d, 2 H, *p*NPS 3,5), 7.27 (s, 10 H, Z C₆H₅), 6.95 (d, 2 H, *p*NPS 2,6), 5.01 (s, 4 H, Z CH₂), 4.89 (m, 1 H, Trp α CH), 4.05 (m, 1 H, Lys α CH), 3.56 (s, 3 H, OMe), 3.30 (m, 2 H, Trp β CH₂), 3.05 (m, 2 H, Lys ε CH₂). Anal. (C₄₀H₄₁N₅O₉S·H₂O) C, H, N, S.

Z-Lys(Z)-Trp(DNPS)-OMe (7): yield 95%; mp 68–70 °C; ¹H NMR (CDCl₃, 90 MHz) δ 9.46 (brs, 1 H, NH^{im}), 8.98 (d, 1 H, DNPS 3), 7.26 (s, 10 H, Z C₆H₅), 6.86 (d, 1 H, DNPS 6), 5.00 (s, 4 H, Z CH₂), 4.90 (m, 1 H, Trp α CH), 4.13 (m, 1 H, Lys α CH), 3.62 (s, 3 H, OMe), 3.40 (m, 2 H, Trp β CH₂), 3.00 (m, 2 H, Lys ε CH₂). Anal. (C₄₃H₄₀N₆O₁₁S) C, H, N, S.

Z-Lys(Z)-Trp(NPS)-OMe (14): yield 99%; foam; ¹H NMR (CDCl₃, 90 MHz) δ 9.16 (brs, 1 H, NH^{im}), 8.20 (dd, 1 H, NPS 3), 7.20 (s, 10 H, Z C₆H₅), 6.63 (dd, 1 H, NPS 6), 5.00 (s, 4 H, Z CH₂), 4.86 (m, 1 H, Trp α CH), 4.03 (m, 1 H, Lys α CH), 3.61 (s, 3 H, OMe), 3.30 (m, 2 H, Trp β CH₂), 3.03 (m, 2 H, Lys ε CH₂). Anal. (C₄₀H₄₁N₅O₉S) C, H, N, S.

Z-Lys(Z)-Trp(AacCmES)-OMe (8). Sulfuryl chloride (0.23 mL) was added, under N₂, to a stirred solution of Ac-Cys-OMe (0.5 g, 2.8 mmol) in dry methylene chloride (30 mL). After the addition was complete, the solution was stirred for 5 min and added to a suspension of 3 (0.75 g, 1.2 mmol) in dry methylene chloride (20 mL). The mixture was stirred for 1 h, and the solvent was removed in vacuo. The residue was purified by preparative TLC using EtOAc/hexane (2:1) to give 8 (0.72 g, 75%): mp 65–67 °C (from EtOAc/hexane); ¹H NMR (CDCl₃, 90 MHz) δ 9.95 (brs, 1 H, NH^{im}), 7.30 (s, 10 H, Z C₆H₅), 5.06 (s, 4 H, Z CH₂), 4.85 (m, 2 H, Trp α CH, AacCmES CH), 4.15 (m, 1 H, Lys α CH), 3.56 (s, 6 H, OMe and AacCmES CO₂CH₃), 3.30 (m, 2 H, Trp β CH₂), 3.10 (m, 4 H, Lys ε CH₂, AacCmES CH₂), 1.90 (s, 3 H, AacCmES NAc). Anal. (C₄₀H₄₇N₅O₁₀S) C, H, N, S.

Z-Lys(Z)-Trp(APS)-OMe (15). A solution of 14 (1.6 g, 2 mmol) in EtOH (100 mL) was hydrogenated at 45 psi and room temperature in the presence of 10% Pd/C (0.16 g). After 17 h, the catalyst was removed by filtration and the filtrate evaporated to leave a chromatographically homogeneous foam (1.42 g), which

was used for the synthesis of 16 without further purification: ¹H NMR from the recently prepared compound (CDCl₃, 90 MHz) δ 9.10 (s, 1 H, NH^{im}), 7.55–6.60 (m, 4 H, APS 3, 4, 5, 6), 7.28 (s, 10 H, Z C₆H₅), 5.03 (s, 4 H, Z CH₂), 4.93 (m, 1 H, Trp α CH), 4.10 (m, 1 H, Lys α CH), 3.60 (s, 3 H, OMe), 3.36 (m, 2 H, Trp β CH₂), 3.00 (m, 2 H, Lys ε CH₂).

Z-Lys(Z)-Trp(AacPS)-OMe (16). A solution of 15 (1.1 g, 1.5 mmol) in dry methylene chloride (60 mL) cooled at –20 °C was treated with acetyl chloride (0.11 mL, 1.5 mmol) and triethylamine (0.17 mL, 1.5 mmol). The mixture was stirred at –20 °C for 2 h and evaporated under reduced pressure and the residue chromatographed on a silica gel column with EtOAc/hexane (2:1) to give 16 (1.14 g, 98%) as a white foam: ¹H NMR (CDCl₃, 90 MHz) δ 9.06 (s, 1 H, NH^{im}), 7.80–6.60 (m, 4 H, AacPS 3, 4, 5, 6), 7.30 (s, 10 H, Z C₆H₅), 5.00 (s, 4 H, Z CH₂), 4.86 (m, 1 H, Trp α CH), 4.03 (m, 1 H, Lys α CH), 3.56 (s, 3 H, OMe), 3.33 (m, 2 H, Trp β CH₂), 3.03 (m, 2 H, Lys ε CH₂), 2.06 (s, 3 H, AacPS NHAc). Anal. (C₄₂H₄₅N₅O₉S) C, H, N, S.

2-(*tert*-Butylsulfonyl)tryptophan Methyl Ester [Trp(*t*-BuS)-OMe] (19). 2-Methyl-2-propanethiol (0.2 mL, 1.9 mmol) was added to a solution of the hexahydropyrrolo[2,3-*b*]indole derivative 18¹⁶ (0.45 g, 1.9 mmol) in 25% TFA (2.5 mL). The mixture was stirred at room temperature for 18 h and evaporated, and the residue was chromatographed on a silica gel column with CHCl₃/MeOH (18:1) to afford 19 (0.35 g, 56%): mp 127–128 °C (from CHCl₃/hexane); ¹H NMR (Me₂SO-*d*₆, 90 MHz) δ 7.70–6.90 (m, 4 H, indole 4, 5, 6, 7), 3.90 (m, 1 H, Trp α CH), 3.46 (s, 3 H, OMe), 3.27 (m, 2 H, Trp β CH₂), 1.26 (s, 9 H, *t*-Bu). Anal. (C₁₆H₂₂N₂O₂S) C, H, N, S.

2-[(2-Carbomethoxyethyl)sulfonyl]tryptophan Methyl Ester [Trp(CmES)-OMe] (20). Methyl 3-mercaptopropionate (0.52 g, 4.3 mmol) was added to solution of the hexahydropyrrolo[2,3-*b*]indole derivative 18¹⁶ (1.0 g, 4.3 mmol) in 25% TFA (10 mL). The mixture was stirred at room temperature for 20 h and evaporated, and the residue was chromatographed on a silica gel column with CHCl₃/MeOH (9:1) to give 20 (1.15 g, 80%): mp 111–112 °C; ¹H NMR (Me₂CO-*d*₆, 90 MHz) δ 7.60–7.00 (m, 4 H, indole 4, 5, 6, 7), 4.40 (m, 1 H, Trp α CH), 3.61 and 3.58 (2 s, 6 H, OMe and CmES CO₂CH₃), 3.53 (m, 2 H, Trp β CH₂), 3.08 (t, 2 H, CmES SCH₂), 2.56 (t, 2 H, CmES CH₂CO₂CH₃). Anal. (C₁₆H₂₀N₂O₄S) C, H, N, S.

Z-Lys(Z)-Trp(*t*-BuS)-OMe (21). Z-Lys(Z)-OSu¹⁸ was added to a solution of 19 (0.3 g, 1.0 mmol) in dry THF (3 mL) (0.5 g, 1.0 mmol), and the mixture was stirred at room temperature for 24 h. Evaporation of the solvent left a residue, which was purified by preparative TLC with EtOAc/hexane (1:1) to provide 21 (0.45 g, 64%) as a white foam: ¹H NMR (CDCl₃, 90 MHz) δ 7.33 (s, 10 H, Z C₆H₅), 4.80 (m, 1 H, Trp α CH), 4.13 (m, 1 H, Lys α CH), 3.40 (m, 2 H, Trp β CH₂), 3.63 (s, 3 H, OMe), 3.07 (m, 2 H, Lys ε CH₂), 1.30 (s, 9 H, *t*-Bu). Anal. (C₃₆H₄₆N₄O₇S) C, H, N, S.

Z-Lys(Z)-Trp(CmES)-OMe (22). *N*-Hydroxysuccinimide (0.18 g, 1.5 mmol) and DCC (0.32 g, 1.5 mmol) were added to a solution of Z-Lys(Z)-OH (0.64 g, 1.5 mmol) in dry THF (10 mL). When the complete formation of Z-Lys(Z)-OSu was detected by analytical TLC, compound 20 (0.55 g, 1.5 mmol) and Et₃N (0.2 mL, 1.5 mmol) were added. After 2 h at room temperature, DCU was filtered off and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel using EtOAc/hexane (2:1) to give 22 (0.96 g, 84%) as a syrup, which crystallized on standing: mp 58 °C; ¹H NMR (CDCl₃, 90 MHz) δ 9.06 (brs, 1 H, NH^{im}), 7.30 (s, 10 H, Z C₆H₅), 5.05 (s, 4 H, Z CH₂), 4.85 (m, 1 H, Trp α CH), 4.10 (m, 1 H, Lys α CH), 3.60 (2 s, 6 H, OMe and CmES CO₂CH₃), 3.31 (m, 2 H, Trp β CH₂), 3.10 (m, 2 H, Lys ε CH₂), 2.95 (t, 2 H, CmES SCH₂), 2.50 (t, 2 H, CmES CH₂CO₂CH₃). Anal. (C₃₈H₄₄N₄O₉S) C, H, N, S.

Removal of the Z Groups. General Procedures. Method A. A solution of BTFA in TFA (6 equiv) was added to a cooled solution (0 °C) of the protected dipeptide (1 mmol) in TFA (1 mL), and the mixture was stirred at room temperature for ~20 h. Removal of the solvent and purification of the residue on a silica gel column using CHCl₃/MeOH (9:1) gave the corresponding unprotected dipeptides. Yields, physical characteristics, and UV data of compounds 9–11, obtained by this method, are recorded in Table I. ¹H NMR data are recorded in Table II.

Method B. Me₃SiI (0.4 mL) was added to a stirred solution of the protected dipeptide (1 mmol) in dry acetonitrile (5 mL).

After 15 min, MeOH (10 mL) was added and the mixture was stirred for an additional 15 min. Removal of the solvent left a residue, which was purified on a silica gel column using $\text{CHCl}_3/\text{MeOH}$ (6:1) to give the corresponding unprotected dipeptides. Yields, physical characteristics, and UV data of compounds 12, 13, 17, 23, and 24, obtained by this method, are recorded in Table I. ^1H NMR data of 12 and 17 are recorded in Table II, while those of 13, 23, and 24 are in Table III.

Analgesia Assay. Analgesia was evaluated in male ICR Swiss albino mice weighing 20–25 g by means of the tail-flick test carried out in the manner described by Nott,¹⁹ using a cutoff time of 10 s. The pain reaction was recorded 30 min before the administration of any drug or saline and at various times later. The control reaction time was in the range of 1.8–2 s. The peptides were dissolved in 0.01 N HCl, neutralized with 0.1 M NaOH, and

injected intracerebroventricularly into conscious animals at a constant volume of 5 μL . The Student's *t* test was used for statistical comparisons.

Acknowledgment. We thank the Consejo Superior de Investigaciones Científicas and the Comisión Asesora de Investigación Científica y Técnica for financial support.

Registry No. 2, 109064-70-2; 3, 109064-71-3; 4, 109064-72-4; 5, 109064-73-5; 6, 109064-74-6; 7, 109064-75-7; 8, 109064-76-8; 9, 109064-77-9; 10, 109064-78-0; 11, 109064-79-1; 12, 109064-80-4; 13, 109064-81-5; 14, 109064-82-6; 15, 109064-83-7; 16, 109064-84-8; 17, 109064-85-9; 18, 51440-62-1; 19, 109064-86-0; 20, 109064-87-1; 21, 109064-88-2; 22, 109064-89-3; 23, 109064-90-6; 24, 109064-91-7; (*o*-MeO₂CC₆H₄S)₂, 5459-63-2; CmPS-Cl, 78880-71-4; PS-Cl, 931-59-9; pNPS-Cl, 937-32-6; DNPS-Cl, 528-76-7; NPS-Cl, 7669-54-7; AcCysOMe, 7652-46-2; *t*-BuSH, 75-66-1; HSCH₂CH₂CO₂Me, 2935-90-2; ZLys(Z)OSu, 21160-83-8; ZLys(Z)OH, 405-39-0; HONSu, 6066-82-6.

(19) Nott, M. V. *Eur. J. Pharmacol.* 1968, 5, 93.

5-Acyl-3-substituted-benzofuran-2(3H)-ones as Potential Antiinflammatory Agents

Jiban K. Chakrabarti,* Richard J. Eggleton, Peter T. Gallagher, Janette Harvey, Terence A. Hicks, E. Ann Kitchen, and Colin W. Smith

Lilly Research Centre Limited, Eli Lilly and Company, Windlesham, Surrey GU20 6PH, England.

Received September 15, 1986

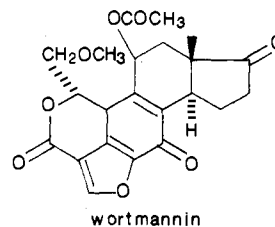
A series of 5-acyl-3-substituted-benzofuran-2(3H)-ones and their respective ring-opened *o*-hydroxy acids were synthesized. The antiinflammatory activity was evaluated in terms of their ability to improve adjuvant induced arthritis in rats. Their effect on the production of both cyclooxygenase (CO) and lipoxygenase (LO) metabolites of arachidonic acid in guinea pig peritoneal polymorphonuclear neutrophils (PMNs) was also examined. No correlation between the antiinflammatory activity and increasing stability of the lactones could be found. The degree of activity in general shown by the benzofuranones was similar to that of their corresponding *o*-hydroxy acids. This, coupled with the evidence from studies on opening of the lactone ring, suggests an *in vivo* transformation of the former into the latter. Benzofuranones displayed a dual inhibition of CO and LO products, while a moderate reduction in CO metabolites was shown by their acids.

In a search for an effective disease-modifying agent, a considerable amount of research has been done over the last decade to understand the mode of action of the non-steroidal antiinflammatory drugs (NSAIDs). Antiinflammatory activity has been associated with the inhibition of prostaglandin synthetase¹ (cyclooxygenase), stabilization of lysosomal membranes,² suppression of mononuclear leucocyte migration,³ and other cellular and biochemical events. These studies in general suggest the multiplicity of actions of NSAIDs. Clinical pathology in chronic inflammatory diseases, e.g., rheumatoid arthritis, indicates the presence of an active immunological response. It is believed that disease-modifying agents interfere with this aberrant immune response by correcting inflammatory cell functions (T-cells, macrophages).

Recently a number of sesquiterpene lactones (e.g., helenalin) and related compounds have been reported⁴ to possess very potent antiinflammatory activity. The mode

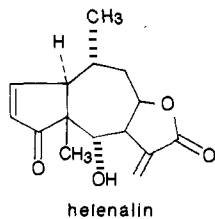
of action of these lactones appeared to be at multiple sites. They inhibited lysosomal enzyme activity, suppressed stimulated migration of human polymorphonuclear neutrophils (PMNs), and inhibited delayed hypersensitivity reactions. The 2-methylene γ -lactone skeleton has been associated with their activity.

Recent work⁵ on potent antiinflammatory mold metabolites, e.g., wortmannin, suggested that the intact furan ring was essential for activity. Following this lead, Close



et al.⁶ have developed a series of 2,3-dihydrobenzofuran-2-ones. Some of them with an attached cyclohexyl or phenyl group showed strong antiinflammatory activity in rats. These were also potent inhibitors of prostaglandin synthesis (*in vitro*). It was, however, not determined whether these lactones were active *per se* or as prodrugs.

A notable variation of the linear biaryl arrangement would be to attach the second aryl group through an angular carbonyl linkage as envisaged in structure A. This



(1) Vane, J. R. *Nature (London)*, New Biol. 1971, 231, 232.

(2) Ignarro, L. J. *Biochem. Pharmacol.* 1971, 20, 2847.

(3) Di Rosa, M.; Papadimitriou, J. M.; Willoughby, D. A. *J. Pathol.* 1971, 105, 239.

(4) Hall, I. H.; Starnes, C. O., Jr.; Lee, K. H.; Waddell, T. G. *J. Pharm. Sci.* 1980, 69, 537.

(5) Wiesinger, D.; Gubler, H. U.; Haefliger, W.; Hauser, D. *Experientia* 1974, 30, 135.

(6) Close, A.; Haefliger, W.; Hauser, D.; Gubler, H. U.; Dewald, B.; Baggiolini, M. *J. Med. Chem.* 1981, 24, 1465.