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. ($C_{24}H_{28}O_3$) C, H.

3-(17β-Hydroxy-6β,7β:15β,16β-bis(methylene)-3-oxoandrosta-1,4,11-trien-17α-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).

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Analgesic Dipeptide Derivatives. 3. Synthesis and Structure–Activity Relationships of *o*-Nitrophenyl-Modified Analogues of the Analgesic Compound H-Lys-Trp(NPS)-OMe[1]

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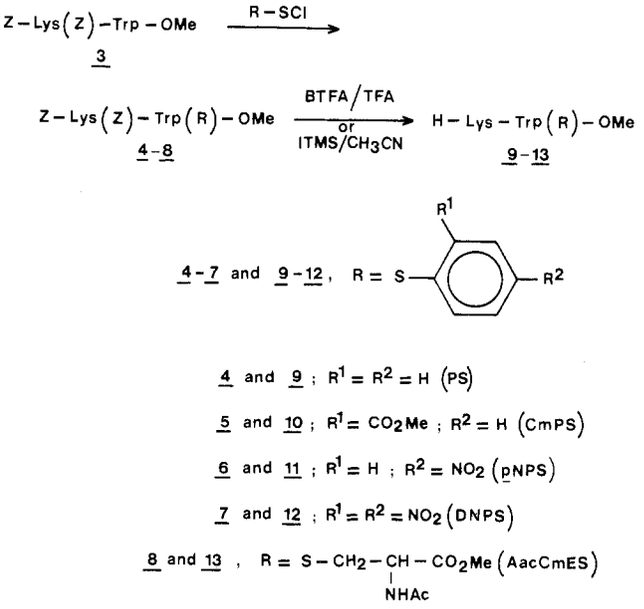
A series of analogues of the analgesic dipeptide derivative H-Lys-Trp(NPS)-OMe has been designed to determine the influence of the (2-nitrophenyl)sulfenyl (NPS) moiety on the activity. The syntheses and antinociceptive effects of these analogues of general formula H-Lys-Trp(R)-OMe [R = phenylsulfenyl (PS) (**9**); R = (2-carbomethoxyphenyl)sulfenyl (CmPS) (**10**); R = (4-nitrophenyl)sulfenyl (*p*NPS) (**11**); R = (2,4-dinitrophenyl)sulfenyl (DNPS) (**12**); R = [2-(acetylamino)-2-carbomethoxyethyl]sulfenyl (AacCmES) (**13**); R = [2-(acetylamino)phenyl]sulfenyl (AacPS) (**17**); R = *tert*-butylsulfenyl (*t*-BuS) (**23**); R = (2-carbomethoxyethyl)sulfenyl (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-(sulfenyl)tryptophan derivatives, which on treatment with boron–tris(trifluoroacetate)/trifluoroacetic acid or trimethylsilyl iodide in acetonitrile (Me_3SiI/CH_3CN) provided **9–13**, respectively. Sulfenylation 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*-butylsulfenyl)- and 2-[(2-carbomethoxyethyl)sulfenyl]tryptophan methyl ester, obtained by reaction of methyl 3a-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 phenylsulfenyl or acyclic sulfenyl 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)sulfenyl] (**1**) exhibited a naloxone-reversible analgesia in mice, when administered intracerebroventricularly, comparable with that of the enkephalin analogue D-Ala[2]-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 phenylsulfenyl or an acyclic sulfenyl moiety. In the first case, the *o*-nitrophenyl portion of **2** has been modified by

Scheme I



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‡ Instituto Cajal.

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

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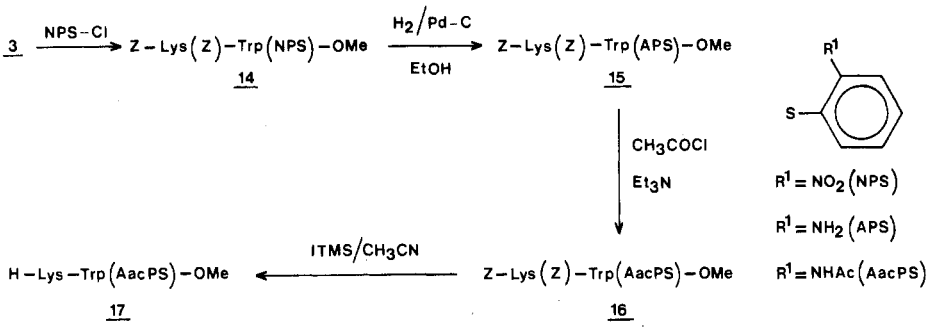
Scheme II

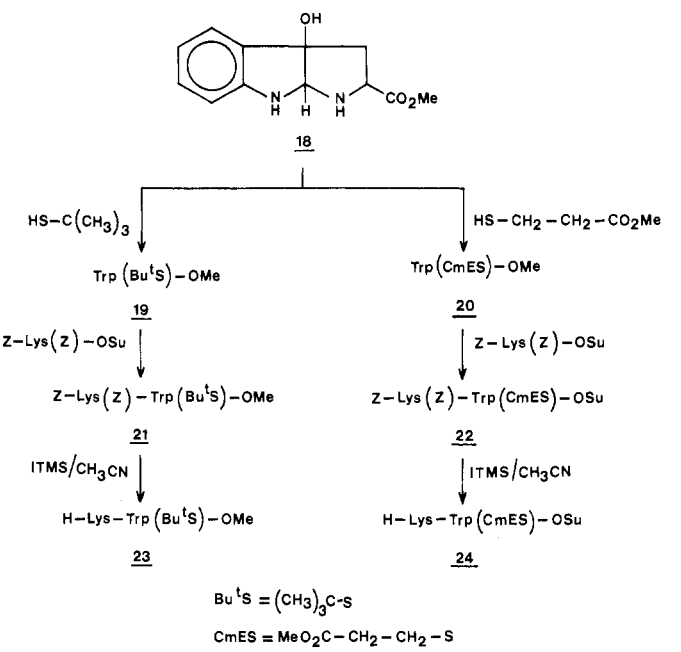


changing either the NO_2 group or its position.

Chemistry

It is known that sulfenyl 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 phenylsulfenyl chloride (PS-Cl), (2-carbomethoxyphenyl)sulfenyl chloride (CmPS-Cl),[6] (4-nitrophenyl)sulfenyl chloride (*p*NPS-Cl), and (2,4-dinitrophenyl)sulfenyl chloride (DNPS-Cl) in dry 1 N HCl in dioxane afforded the corresponding 2-sulfenyltryptophan 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 sulfenylating 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 disulfide[9] using fuming H_2SO_4 as catalyst. [2-(Acetylamino)-2-carbomethoxyethyl]sulfenyl chloride (AacCmES-Cl)[6] was generated by treatment of Ac-Cys-OMe with sulfuryl chloride in methylene chloride.[10] This sulfenylating agent in situ reacted with the protected dipeptide **3** to give the corresponding Trp(AacCmES) derivative **8**. Although all these sulfenylation 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 (Me_3SiI/CH_3CN)[12] for **7** and **8** provided the corresponding deprotected analogues H-Lys-Trp(R)-OMe **9–13**.

A similar sulfenylation reaction of the protected dipeptide **3**, using (*o*-nitrophenyl)sulfenyl chloride (NPS-Cl), gave the NPS derivative **14**, which, upon hydrogenolysis of the NO_2 group at room temperature and 45 psi employing 10% Pd/C as catalyst, readily provided the corresponding (aminophenyl)sulfenyl (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(N_3)-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

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(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)sulfenyl has been used, instead of [(methoxycarbonyl)phenyl]sulfenyl, with the end of abbreviating this group as CmPS, since CPS has been previously used to represent the free acid [(carboxyphenyl)-sulfenyl group] (Parikh, I.; Omenn, G. S. *Biochemistry* **1971**, *10*, 1173). Accordingly, CmES has been employed to represent (carbomethoxyethyl)sulfenyl. In order to employ homogeneous abbreviations and nomenclature, the $MeO_2CCH(NHAc)CH_2S$ 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.
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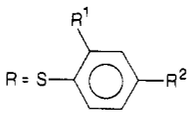
Table I. Physical Properties and UV Data of H-Lys-Trp(R)-OMe

no.	R	yield,[a] %	mp,[b] °C	formula[c]	scheme	UV λ_{max} (EtOH), nm (ϵ)
9	PS	78	218–219	$C_{24}H_{30}N_4O_3S$	I	286 (18 800)
10	CmPS	99	112–113	$C_{26}H_{32}N_4O_5S$	I	290 (7800)
11	*p*NPS	74	82–84	$C_{24}H_{29}N_5O_5S$	I	292 (10 800), 320 (8450)
12	DNPS	92	foam	$C_{24}H_{28}N_6O_7S$	I	275 (17 200), 290 (13 400)
13	AacCmES	84	200 dec	$C_{24}H_{35}N_5O_6S$	I	290 (3950)
17	AacPS	84	198 dec	$C_{26}H_{33}N_5O_4S$	II	284 (3800)
23	*t*-BuS	81	foam	$C_{22}H_{34}N_4O_3S\cdot H_2O$	III	285 (8100), 290 (8350)
24	CmES	85	foam	$C_{22}H_{32}N_4O_5S$	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 Me_4Si) of H-Lys-Trp(R)-OMe in Me_2SO-d_6 at 300 MHz



			phenylsulfenyl protons					Trp			Lys	
no.	R^1	R^2	H-2	H-3	H-4	H-5	H-6[a]	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_2Me	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	*b*	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 H_2O signal contained in Me_2SO-d_6.

Table III. Partial Proton Chemical Shifts (ppm from Me_4Si) of H-Lys-Trp(R)-OMe in Me_2SO-d_6 at 300 MHz

R = S–R^1

		Trp			Lys			
no.	R^1	H-α	H′-β	H″-β	H-α	H-ϵ	OMe	R^1 protons
13	$CH_2CH(NHAc)CO_2Me$	4.58	3.30	3.14	3.72	2.80	3.54	4.28 (CH_2); 3.60 (CO_2Me); 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	$(CH_2)_2CO_2Me$	4.53	3.27	3.19	3.67	2.77	3.46	3.53 (CO_2Me); 3.04 (SCH_2); 2.50 (CH_2CO_2Me)

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 Me_3SiI/CH_3CN, 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 CO_2Me 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 CmES[6] 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 Me_3SiI/CH_3CN, 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-(*p*NPS)-containing peptides.[4] Evidence for the presence of the PS, CmPS, *p*NPS, 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

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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*-NO_2 and *o*-CO_2Me 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 [^{3}H]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 NO_2 substituent of **2** was replaced by the NHAc group (see **17**). The presence of an additional NO_2 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 NO_2 and CO_2Me 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 *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 CO_2Me 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 ^{1}H 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 NO_2 or CO_2Me 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.

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	*b*	*b*	*b*
12	0.1	21 ± 16	23 ± 15	28 ± 12
	0.5	*b*	*b*	*b*
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

[a] Results are the means ± SE obtained with groups of 10–12 mice. (*) Significant change ($p < 0.05$ or better, Student's t test). [b] Signs neurotoxicity consisting of motor incoordination, signs of respiratory disturbances, and barrel rotations.

Chemical Methods

Melting points were measured with a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 or a Varian XL-300 spectrometer operating at 90 or 300 MHz, respectively, using Me_4Si as internal standard and $CDCl_3$ or Me_2SO-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 $60F_{254}$ (Merck). Preparative layer chromatography was performed on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF_{254} (Merck). Silica gel 60 (230–400 mesh) (Merck) was used for column chromatography. Compounds were detected with UV light (254 nm). (o-Nitrophenyl)sulfenyl chloride (NPS-Cl) and (2,4-dinitrophenyl)sulfenyl chloride (DNPS-Cl) were from Sigma. Phenylsulfenyl chloride (PS-Cl), (4-nitrophenyl)sulfenyl chloride (pNPS-Cl), and [2-(acetylamino)-2-carbomethoxyethyl]sulfenyl chloride (AacCmES-Cl) were prepared according to the literature procedures.[7,8,10]

(2-Carbomethoxyphenyl)sulfenyl Chloride (CmPS-Cl). A suspension of methyl 2,2′-dicarbomethoxydiphenyl disulfide[10] (0.4 g, 1.2 mmol) in 1,2-dichloroethane (20 mL) containing 1 drop of fuming H_2SO_4 was stirred at room temperature while Cl_2 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.

Phenylsulfenylation of Z-Lys(Z)-Trp-OMe (3). General Procedure. The sulfenyl chloride derivative (1.5 mmol) was added to a solution of the dipeptide derivative **3**[5] (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; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 8.60 (brs, 1 H, NH^{in}), 7.60–6.90 (m, 5 H, PS 2, 3, 4, 5, 6), 7.30 (s, 10 H, Z C_6H_5), 5.00 (s, 4 H, Z CH_2), 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 CH_2), 3.03 (m, 2 H, Lys ε CH_2). Anal. ($C_{40}H_{42}N_4O_7S$) C, H, N, S.

Z-Lys(Z)-Trp(CmPS)-OMe (5): yield 86%; mp 73–74 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 8.75 (brs, 1 H, NH^{in}), 7.95 (dd, 1 H, CmPS 3), 7.34 (s, 10 H, Z C_6H_5), 6.60 (dd, 1 H, CmPS 6), 5.00 (s, 4 H, Z CH_2), 4.87 (m, 1 H, Trp α CH), 4.05 (m, 1 H, Lys α CH), 3.87 (s, 3 H, CmPS CO_2CH_3), 3.61 (s, 3 H, OMe), 3.30 (m, 2 H, Trp β CH_2), 3.05 (m, 2 H, Lys ε CH_2). Anal. ($C_{42}H_{44}N_4O_9S$) C, H, N, S.

Z-Lys(Z)-Trp(pNPS)-OMe (6): yield 94%; mp 70–72 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 9.00 (brs, 1 H, NH^{in}), 7.93 (d, 2 H, pNPS 3,5), 7.27 (s, 10 H, Z C_6H_5), 6.95 (d, 2 H, pNPS 2, 6), 5.01 (s, 4 H, Z CH_2), 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 CH_2), 3.05 (m, 2 H, Lys ε CH_2). Anal. ($C_{40}H_{41}N_5O_9S\cdot H_2O$) C, H, N, S.

Z-Lys(Z)-Trp(DNPS)-OMe (7): yield 95%; mp 68–70 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 9.46 (brs, 1 H, NH^{in}), 8.98 (d, 1 H, DNPS 3), 7.26 (s, 10 H, Z C_6H_5), 6.86 (d, 1 H, DNPS 6), 5.00 (s, 4 H, Z CH_2), 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 CH_2), 3.00 (m, 2 H, Lys ε CH_2). Anal. ($C_{43}H_{40}N_6O_{11}S$) C, H, N, S.

Z-Lys(Z)-Trp(NPS)-OMe (14): yield 99%; foam; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 9.16 (brs, 1 H, NH^{in}), 8.20 (dd, 1 H, NPS 3), 7.20 (s, 10 H, Z C_6H_5), 6.63 (dd, 1 H, NPS 6), 5.00 (s, 4 H, Z CH_2), 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 CH_2), 3.03 (m, 2 H, Lys ε CH_2). Anal. ($C_{40}H_{41}N_5O_9S$) C, H, N, S.

Z-Lys(Z)-Trp(AacCmES)-OMe (8). Sulfuryl chloride (0.23 mL) was added, under N_2, 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); ^{1}H NMR ($CDCl_3$, 90 MHz) δ 9.95 (brs, 1 H, NH^{in}), 7.30 (s, 10 H, Z C_6H_5), 5.06 (s, 4 H, Z CH_2), 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 CO_2CH_3), 3.30 (m, 2 H, Trp β CH_2), 3.10 (m, 4 H, Lys ε CH_2, AacCmES CH_2), 1.90 (s, 3 H, AacCmES NAc). Anal. ($C_{40}H_{47}N_5O_{10}S$) 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: ^{1}H NMR from the recently prepared compound ($CDCl_3$, 90 MHz) δ 9.10 (s, 1 H, NH^{in}), 7.55–6.60 (m, 4 H, APS 3, 4, 5, 6), 7.28 (s, 10 H, Z C_6H_5), 5.03 (s, 4 H, Z CH_2), 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 CH_2), 3.00 (m, 2 H, Lys ε CH_2).

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: ^{1}H NMR ($CDCl_3$, 90 MHz) δ 9.06 (s, 1 H, NH^{in}), 7.80–6.60 (m, 4 H, AacPS 3, 4, 5, 6), 7.30 (s, 10 H, Z C_6H_5), 5.00 (s, 4 H, Z CH_2), 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 CH_2), 3.03 (m, 2 H, Lys ε CH_2), 2.06 (s, 3 H, AacPS NHAc). Anal. ($C_{42}H_{45}N_5O_8S$) C, H, N, S.

2-(*tert*-Butylsulfenyl)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 **18**[16] (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 $CHCl_3$/MeOH (18:1) to afford **19** (0.35 g, 56%): mp 127–128 °C (from $CHCl_3$/hexane); ^{1}H NMR (Me_2SO-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 CH_2), 1.26 (s, 9 H, *t*-Bu). Anal. ($C_{16}H_{22}N_2O_2S$) C, H, N, S.

2-[(2-Carbomethoxyethyl)sulfenyl]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 **18**[16] (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 $CHCl_3$/MeOH (9:1) to give **20** (1.15 g, 80%): mp 111–112 °C; ^{1}H NMR (Me_2CO-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 CO_2CH_3), 3.53 (m, 2 H, Trp β CH_2), 3.08 (t, 2 H, CmES SCH_2), 2.56 (t, 2 H, CmES $CH_2CO_2CH_3$). Anal. ($C_{16}H_{20}N_2O_4S$) C, H, N, S.

Z-Lys(Z)-Trp(*t*-BuS)-OMe (21). Z-Lys(Z)-OSu[18] 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: ^{1}H NMR ($CDCl_3$, 90 MHz) δ 7.33 (s, 10 H, Z C_6H_5), 4.80 (m, 1 H, Trp α CH), 4.13 (m, 1 H, Lys α CH), 3.40 (m, 2 H, Trp β CH_2), 3.63 (s, 3 H, OMe), 3.07 (m, 2 H, Lys ε CH_2), 1.30 (s, 9 H, *t*-Bu). Anal. ($C_{36}H_{46}N_4O_7S$) 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 Et_3N (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; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 9.06 (brs, 1 H, NH^{in}), 7.30 (s, 10 H, Z C_6H_5), 5.05 (s, 4 H, Z CH_2), 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 CO_2CH_3), 3.31 (m, 2 H, Trp β CH_2), 3.10 (m, 2 H, Lys ε CH_2), 2.95 (t, 2 H, CmES SCH_2), 2.50 (t, 2 H, CmES $CH_2CO_2CH_3$). Anal. ($C_{38}H_{44}N_4O_9S$) 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 $CHCl_3$/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. ^{1}H NMR data are recorded in Table II.

Method B. Me_3SiI (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 $CHCl_3$/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.

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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; $(o\text{-}MeO_2CC_6H_4S)_2$, 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; $HSCH_2CH_2CO_2Me$, 2935-90-2; ZLys(Z)OSu, 21160-83-8; ZLys(Z)OH, 405-39-0; HONSu, 6066-82-6.

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

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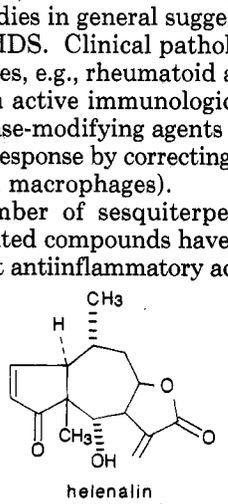
A series of 5-acyl-3-substituted-benzofuran-2(3*H*)-ones and their respective ring-opened *o*-hydroxy acids were synthesized. The antiinflammatory activity was evaluated in terms of their ability to improve adjuvant induced arthritis in rats. Their effect on the production of both cyclooxygenase (CO) 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.

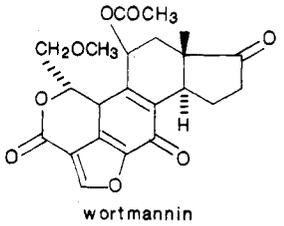
In a search for an effective disease-modifying agent, a considerable amount of research has been done over the last decade to understand the mode of action of the nonsteroidal antiinflammatory drugs (NSAIDS). Antiinflammatory activity has been associated with the inhibition of prostaglandin synthetase[1] (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 reported[4] 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.



helenalin

Recent work[5] 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.



wortmannin

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

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