methoxyphenyl)-1-pentene, 52093-69-3; 6-(2-methoxyphenyl)-1-hexene, 89789-98-0; allylbenzene, 300-57-2; 4-phenyl-1-butene, 768-56-9; 5-phenyl-1-pentene, 1075-74-7; 4-(4-methoxyphenyl)-1-butene, 20574-98-5; epichlorohydrin, 106-89-8; 1-chloro-4-(2-methylphenyl)-2-butanol, 79407-26-4; 1,2-bis(2-methylphenyl)-ethane, 952-80-7; *p*-anisyl chloride, 824-94-2; allyl bromide, 106-95-6; 2,3-dihydro-2,2-dimethyl-6-(allyloxy)-4*H*-1,3-benzoxazine, 76813-99-5.

A Photoaffinity Reagent To Label the Opiate Receptors of Guinea Pig Ileum and Mouse Vas Deferens

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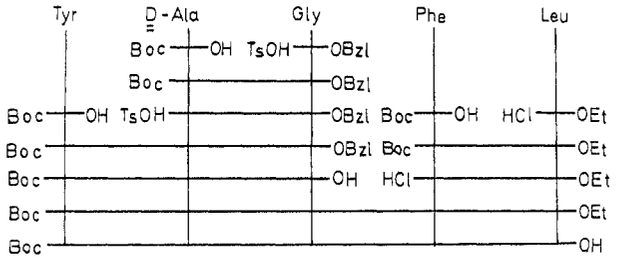
An enkephalin derivative, [D-Ala^2,Leu^5]enkephalin *N*-[(2-nitro-4-azidophenyl)amino]ethylamide, has been synthesized as a photoaffinity label for the opiate receptor. This compound retains the full biological activity of [D-Ala^2,Leu^5]enkephalin in guinea pig ileum and mouse vas deferens tests with IC_{50} values of 4.4 and 2.6 nM, respectively, and inhibits the binding of [^{3}H]naloxone to rat brain membrane preparation with an IC_{50} value of 2.5 nM. Photolysis of a muscle strip of the guinea pig ileum or of the mouse vas deferens in the presence of the peptide derivative caused irreversible inhibition of electrically stimulated contractions with high efficiencies (80 and 66%, respectively), while the inhibitory effect in the dark was fully reversed by washing. This irreversible inhibition during photolysis was completely prevented by the presence of [D-Ala^2,Leu^5]enkephalin. These results demonstrate that [D-Ala^2,Leu^5]-enkephalin *N*-[(2-nitro-4-azidophenyl)amino]ethylamide is a prominent candidate as a photoaffinity label for the opiate receptor.

The discovery of enkephalins[1] and endorphins,[2-4] endogenous peptide ligands with opiate properties, sparked the enormous research activity that has characterized the opiate field in recent years. Other new endogenous peptides have been discovered subsequently.[5-9] At present the opioid peptides can be classified into the following three groups: endorphins, enkephalins, and dynorphins/neo-endorphins; the occurrence of these three sets of opioids derived from three different genes is clearly established.[10-13] A number of reports on subcellular distributions of the opioid peptides and their receptors have been published.[14-17] The observations of heterogeneous binding of opioid ligands to receptors[18,19] raise some intriguing questions on whether multiple classes of opioid receptors (at least three types designated μ, δ, and κ[18]) correlate with the products of the three opioid peptide genes.

Little significant progress, however, has been made with respect to receptors at the molecular level. It is still unclear whether these various receptor forms represent different molecular entities or result from conformational changes of a single receptor species. Isolation and biochemical characterization of the cell-surface receptor are thus of prime importance. Several attempts along this line have been reported, such as solubilization of the receptor[20-23] and partial purification by affinity chromatography.[24]

Special difficulties in studying opiate receptors lie in the fact that the receptors are present in very small quantities (ca. 10 fmol/mg of guinea pig brain) and are membrane-bound proteins sensitive to protelytic enzymes and detergents. One promising approach is the use of affinity labels, particularly the photoaffinity labeling technique.[25,26] We have shown the usefulness of this technique in the labeling of an enzyme active site.[27] Several photoaffinity compounds for the opiate receptors, based on opiate drugs[28-30] or enkephalins,[31-34] have been synthesized and applied. However, for the wavelength used (254 nm) it has been shown recently that this short-wavelength UV light causes rapid destruction of the opiate binding activity of the receptor.[35] Accordingly, we have designed a pho-

Scheme I. A Synthetic Route for N^{α}-Boc-[D-Ala^2,Leu^5]enkephalin



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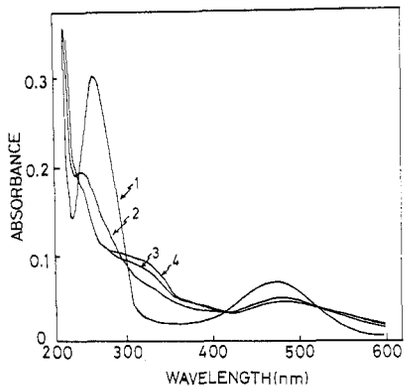
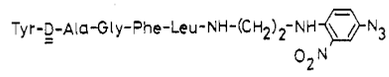


Figure 1. Absorption spectra of ENAPE before and after photolysis (flash). ENAPE (10 μM) in water was photolyzed in the Pyrex organ bath at a distance of 5 cm from the lamp: 1, before photolysis; 2, 50 flashes; 3, 100 flashes; 4, 150 flashes.

toaffinity compound for the receptor, [D-Ala^2,Leu^5]enkephalin *N*-[(2-nitro-4-azidophenyl)amino]ethylamide, con-



taining a chromophore of adequate absorption maximum at long wavelength: This chromophore is attached to the [D-Ala^2,Leu^5]enkephalin molecule, which is known to be resistant to peptidase degradation in biological preparations.[36]

Table I. Inhibitory Effects of the Peptides and Naloxone on the Binding of [^{3}H]Naloxone (2 nM) in the Preparation of Rat Brain Membrane

compound	IC_{50},[a] nM	rel potency[b]
naloxone	6.39 ± 1.46 (7)	18.5
[Leu^5]enkephalin	118 ± 27.3 (4)	1
DALE	30.0 ± 7.64 (3)	3.9
ENAPE	2.52 ± 1.00 (3)	46.8
β-endorphin	8.43 ± 2.79 (3)	14.0

[a] The values are the means plus or minus standard error; the number of determinations is given in parentheses. [b] Relative potency was calculated from the ratio of the IC_{50} of [Leu^5]-enkephalin to that of the compound.

Table II. Inhibitory Effects of Enkephalin and Its Derivatives on the Guinea Pig Ileum (GPI) and Mouse Vas Deferens (MVD) Assays

peptide	IC_{50},[a] nM	
	GPI	MVD
[Leu^5]enkephalin	120 ± 10 (50)	17.0 ± 5.5 (50)
DALE	5.23 ± 0.59 (3)	2.88 ± 0.90 (3)
ENAPE	4.35 ± 1.57 (3)	2.59 ± 0.97 (2)

[a] The values are the means plus or minus standard error; the number of determinations is given in parentheses.

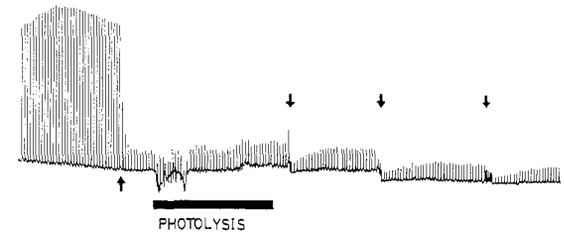


Figure 2. Inhibition of the electrically induced contractions of GPI by photolysis in the presence of ENAPE. The GPI in the Pyrex organ bath was photolyzed with ENAPE (100 nM) at room temperature in modified Krebs solution (see text). The "up" arrow indicates the addition of ENAPE, and the "down" arrows show replacements of the bath solution.

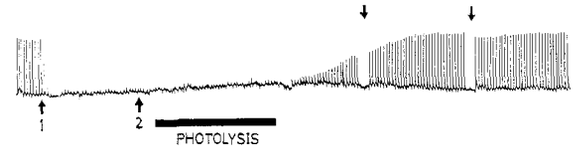


Figure 3. Prevention by DALE of the photochemical inactivation of GPI with ENAPE. GPI in the Pyrex organ bath was photolyzed in the presence of DALE (20 μM) and ENAPE (10 nM) at room temperature in the same solution as in Figure 2. The "up" arrows indicate the addition of drugs: 1, DALE; 2, ENAPE. The "down" arrows show replacements of the bath solution.

We report here the synthesis of [D-Ala^2,Leu^5]enkephalin *N*-[(2-nitro-4-azidophenyl)amino]ethylamide (ENAPE),[37] its biological activities, and its application to the photoaffinity labeling of the opiate receptors in the guinea pig

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(37) Abbreviations used follow IUPAC–IUB tentative rules as described in *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations used are as follows: ENAPE, [D-Ala^2,Leu^5]enkephalin *N*-[(2-nitro-4-azidophenyl)amino]ethylamide; DALE, [D-Ala^2,Leu^5]enkephalin; EDC·HCl, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; TsOH, *p*-toluenesulfonic acid; EtOAc, ethyl acetate; GPI, guinea pig ileum; MVD, mouse vas deferens.

ileum and mouse vas deferens.

Results

[D-Ala^2,Leu^5]enkephalin *N*-[(2-Nitro-4-azidophenyl)amino]ethylamide (ENAPE). N^{α}-Boc-[D-Ala^2,Leu^5]enkephalin (**2**) was synthesized by conventional solution methods employing a (3 + 2) fragment condensation (Scheme I). The coupling of **2** with *N*-(2-nitro-4-azidophenyl)ethylenediamine (**1**) was achieved by the use of EDC·HCl in the presence of HOBt. In the absence of HOBt, another undesired product was also detected on TLC. In several trials for deblocking the *tert*-butoxycarbonyl group, the use of HCl/EtOAc removed it effectively. The modified method using trifluoroacetic acid under mild conditions[32] gave several spots on TLC and so was not suitable for this case. The deblocked product was purified by TLC and was obtained as a hydrochloride salt after precipitation with hydrochloric acid.

Absorption spectra of ENAPE before and after photolysis are shown in Figure 1. As expected, ENAPE has a sufficient absorption maximum at long wavelength (445 nm; ϵ 4600 M^{-1} cm^{-1}). Photolysis of the compound in the Pyrex bath, as described in the Experimental Section, was complete after 100 flashes. The absorption peaks at 260 and 455 nm of ENAPE disappeared after photolysis, and a small shoulder appeared at 325 nm.

Opiate Activities of ENAPE. Opiate activities of ENAPE were determined by measuring the binding activity to a rat brain membrane and by bioassay using guinea pig ileum (GPI) or mouse vas deferens (MVD). ENAPE proved to be a potent opiate agonist. It competes with [^{3}H]naloxone for binding to a membrane preparation from rat brain and has higher affinity than that of [Leu^5]enkephalin, [D-Ala^2,Leu^5]enkephalin (DALE), or β-endorphin (Table I). ENAPE was also shown to be biologically active by its inhibition of the electrically stimulated contractions of GPI and MVD. It is as equally active as the parent compound, DALE (Table II).

Inhibition of Electrically Induced Contractions of GPI by Photolysis in the Presence of ENAPE. Photolysis in the presence of ENAPE inhibited the contractions of GPI strips (Figure 2). Inhibition of 53 or 91% was observed at an ENAPE concentration of 10 or 100 nM, respectively. This inhibition was not influenced by replacing the bath solution (washing) within 3 h, while the inhibitory effects of ENAPE in the dark were completely eliminated by washing. In addition, inhibition of contractions by photolyzed ENAPE was reversed by washing: 36 or 27% inhibition at a concentration of 5 or 20 nM photolyzed ENAPE, respectively, was eliminated by replacing the bath solution. These results suggest that photolysis in the presence of ENAPE results in irreversible labeling of the opiate receptors of GPI.

Protection with DALE against Irreversible Inhibition of GPI Contractions by Photolysis in the Presence of ENAPE. Figure 3 shows that 20 μM DALE protects GPI activity against the long-lasting inhibition of contractions by photolysis in the presence of 10 nM ENAPE. DALE (40 μM) also showed the protection effect against the photochemical inhibition with 100 nM ENAPE. Strips exposed to identical treatment without photolysis (in the dark) responded identically to [Leu^5]enkephalin as a control (data not shown). When GPI was photolyzed without ENAPE as another control, 90% of the opiate binding activity was retained.

Photochemical Inhibition of MVD Contractions with ENAPE. Electrically induced contractions of MVD were inhibited by photolysis in the presence of 10 nM ENAPE (Figure 4). The observed inhibition was 76% of the original activity even after the MVD strip was washed with bath solution for 1 h, while the inhibition of ENAPE in the dark was completely removed by the washing procedures. DALE, 10 μM, protected the MVD contraction activity from photochemical inhibition with 10 nM ENAPE. MVD preparations were a little more sensitive to photoirradiation than GPI, but 90% of the activity remained within 1 h even after the irradiation as a control experiment.

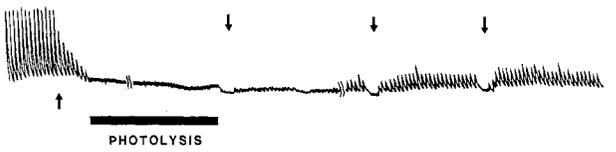

Figure 4. Photochemical inhibition of the electrically induced contractions of MVD in the presence of ENAPE. A MVD strip in the Pyrex organ bath was photolyzed with ENAPE (10 nM) at room temperature in modified Krebs solution. The "up" arrow indicates the addition of ENAPE, and the "down" arrows show replacement of the bath solution.

Discussion

We have synthesized an enkephalin analogue, [D-Ala^2,Leu^5]enkephalin *N*-[(2-nitro-4-azidophenyl)amino]-ethylamide (ENAPE), which is a good candidate for the photochemical labeling of the opiate receptor. Biologically, the compound shows high opiate binding activities by three different assay systems, radio-receptor binding assay to rat brain homegenates and bioassay in GPI and MVD. It is of note that ENAPE retains full opiate activity of DALE, although a photosensitive nitrophenylazido group with relatively large steric requirements was introduced to the carboxyl terminal of DALE. This also suggests that carboxyl-terminal modifications of the enkephalin are not critical for its binding to the receptor.[38]

ENAPE was also well characterized chemically by elemental analysis, amino acid analysis, and several spectrometric determinations. It has a sufficient absorption band at 455 nm to be photolyzed with long-wavelength light, which was confirmed to be nondestructive to the receptor in this work. The irradiation wavelength in photoaffinity labeling is chosen based on two factors: one is absorption regions of the photosensitive group attached to the ligands, and the other is the photosensitivity of the biological system. In general, irradiation at short wavelength (254 nm) can easily activate the photosensitive groups. Therefore, irradiation for a short time is enough to photolyze them to reactive species with a high energy level. In fact, it has been observed that higher labeling efficiencies can be obtained in some cases.[25] In the case of the opiate receptor, however, it was reported that short-wavelength UV light (254 nm) causes rapid and critical loss of binding activity.[35] In order to minimize such damage, we used Xenon flashes and a Pyrex organ bath. Under the irradiation conditions, wavelengths longer than 280 nm are responsible for activating the photosensitive group of ENAPE to the reactive nitrene species.

Efficient irreversible inhibition (91%) of the electrically stimulated contractions of GPI was observed by photolysis in the presence of 100 nM ENAPE (Figure 2). A net inhibition of 80% was achieved after subtracting the 10% loss of activity that occurred by photolysis of the GPI alone. This inhibition of ENAPE was completely prevented in the presence of a competing ligand such as DALE. Therefore, these results demonstrate that the irreversible inhibition by photolysis with ENAPE resulted

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from its specific photolabeling to the opiate receptor. Similar irreversible inhibition (66% net) was observed photochemically in MVD contractions with 10 nM ENAPE (Figure 3). GPI and MVD are reported to contain predominantly μ- and δ-type receptors, respectively,[46,49] although a smaller amount of κ-type receptors can not be excluded. Therefore, the observation that both GPI and MVD were inactivated photochemically with ENAPE suggests the compound does not have a significant preference for either of the two receptor preparations from different sources. Since the binding potential of ENAPE to both receptors is quite similar (Table II), the lack of selectivity of ENAPE is unlikely to result from the photoreaction step but rather from the binding step.

The apparent broad specifity of ENAPE does not seem to be suitable for accomplishing the specific photolabeling of the δ receptor in the MVD preparation because it also contains other types of opioid receptors.[49] On the other hand, ENAPE can be employed in a cultured cell line such as NG 108-15, which contains only δ receptors.[50] Therefore, when these different types of preparations were employed and when the labeled receptor proteins were comparatively analyzed, ENAPE was able to distinguish structual differences in the case of one type of receptor; i.e., that it is composed of different protein subunits than the others. For this purpose, the use of [^{125}I]ENAPE is expected to facilitate detection and isolation of the photolabeled proteins of the receptor; thus, the synthesis of the iodinated derivatives is now in progress.

Experimental Section

The following compounds were obtained from the indicated sources: [Leu^5]enkephalin and β-endorphin, Protein Research Foundation, Osaka; naloxone, Sankyo Co., Tokyo; [^{3}H]naloxone (specific radioactivity 13.2 Ci/mmol), New England Nuclear Co. Melting points were determined with a Yamato MP-1 melting point apparatus and are uncorrected. IR data were recorded on a JASCO IRA-1 spectrometer. The mass spectral data were obtained with a JEOL JMS-300 mass spectrometer or a mass spectrometer in the field-desorption (FD) mode. The absorption spectra were determined in water or in EtOH with a Hitachi UV 200-10 spectrophotometer. Optical rotations were measured with a JASCO DIP-4 polarimeter. Amino acid analysis was determined on a JEOL JLC-6AH amino acid analyzer following hydrolysis in degassed 6 N HCl at 110 °C for 24 h. Molar ratios were referred to Ala (=1.00). Homogeneity of the products was checked on precoated TLC plates (silica gel 60 F254, Merk), using the following solvent systems: CH_2Cl_2/MeOH, 5:1; BuOH/AcOH/H_2O, 4:1:2; EtOAc/pyridine/AcOH/H_2O, 60:20:6:11. TLC of the compounds described below showed homogeneous single spots with UV and/or I_2. The analytical results for elements indicated by their symbols were within ±0.4% of the theoretical values.

Syntheses. All azido derivatives were handled in the dark.

***N*-(2-Nitro-4-azidophenyl)ethylenediamine (1).** This compound was prepared from 4-fluoro-3-nitrophenyl azide by a modification of the reported method.[39] A solution of 4-fluoro-3-nitrophenyl azide (910 mg, 5 mmol) in dry CH_2Cl_2 (10 mL) was added to ethylenediamine (3.0 g, 50 mmol) in dry CH_2Cl_2 (10 mL), and the resulting mixture was stirred at room temperature for 30 min. The mixture was washed with water, and the solution was evaporated to dryness in vacuo. The product was purified by silica gel chromatography (CH_2Cl_2/MeOH, 5:1) and was recrystallized from ether/hexane under nitrogen atmosphere (to prevent the formation of the carbonate salt): yield 77 mg (34%); mp 64–65 °C (lit.[40] 64–68 °C); mass spectrum, *m/e* 222 (M^+); IR spectrum (Nujol), 2120 (N_3) cm^{-1}. Anal. ($C_8H_{10}N_6O_2$) C, H, N.

***N*$^{\alpha}$-(*tert*-Butoxycarbonyl)[D-Ala^2,Leu^5]enkephalin (2).** This peptide was prepared by conventional solution methods (Scheme I). The product was recrystallized from acetone/EtOAc/petroleum ether: mp 145.5–147 °C dec; $[\alpha]^{20}_D$ −14.0 ± 0.4° (*c* 1.00, DMF). Anal. ($C_{34}H_{47}N_5O_9 \cdot H_2O$) C, H, N. Amino acid analysis: Tyr, 1.08; Ala, 1.00; Gly, 0.97; Phe, 1.04; Leu, 0.96. Deblocking of the *tert*-butoxycarbonyl group from **2** by trifluoroacetic acid in the presence of anisole, followed by purification with DEAE-Sephadex A-25 (Pharmacia Fine Chemicals),[41] gave [D-Ala^2,Leu^5]enkephalin (DALE), which was recrystallized from MeOH/ether (47%): mp 164–168 °C dec; $[\alpha]^{20}_D$ +21.6 ± 0.3° (*c* 1.01, MeOH). Amino acid analysis: Tyr, 1.01; Ala, 1.00; Gly, 0.98; Phe, 1.06; Leu, 1.02.

***N*$^{\alpha}$-(*tert*-Butoxycarbonyl)[D-Ala^2,Leu^5]enkephalin *N*-[(2-Nitro-4-azidophenyl)amino]ethylamide (3).** This compound was prepared by the modified procedure reported by Lee et al.[32] A suspension of **2** (670 mg, 1 mmol) in dry acetonitrile (60 mL) was added to the solution of **1** (444 mg, 2 mmol) and HOBt[37] (203 mg, 1.5 mmol) in dry CH_2Cl_2/acetonitrile (1:1, 270 mL), and then EDC·HCl[37] (286 mg, 1.5 mmol) was added to the mixture. The mixture was stirred overnight at room temperature. After filtration of the resulting precipitate, the filtrate was evaporated to dryness in vacuo. The residue was dissolved in EtOAc, and then the solution was washed with water, 1 N HCl, 4% $NaHCO_3$, and saturated NaCl and was dried over $MgSO_4$. The solution was evaporated to dryness in vacuo, then the residue was redissolved in acetone. A reddish amorphous powder was obtained by the addition of ether/petroleum ether to the solution (763 mg, 87%): mp 190 °C dec; IR (Nujol) 2120 (N_3) cm^{-1}. Anal. ($C_{42}H_{55}N_{11}O_{10}$) C, H, N.

[D-Ala^2,Leu^5]enkephalin *N'*-[(2-Nitro-4-azidophenyl)amino]ethylamide (ENAPE). A solution of **3** (87 mg, 0.1 mmol) in EtOAc containing 1 N HCl (4 mL) was stirred for 2 h at room temperature, and then ether was added. The resulting precipitate was filtered off and purified by silica gel TLC (CH_2Cl_2/MeOH, 7:1). The product, a reddish amorphous powder (41 mg, 51%), was precipitated from its solution in MeOH containing 1 N HCl in EtOAc (0.12 mL) by the addition of ether: mp 220 °C dec; IR 2120 (N_3) cm^{-1}; UV λ_{max} (EtOH) 260 nm (ϵ 20 800), 455 (4600); FD mass spectrum, *m/e* 744 (M^+). Anal. ($C_{37}H_{47}N_{11}O_8 \cdot HCl \cdot H_2O$) C, H, N. Amino acid analysis: Tyr, 0.97; Ala, 1.00; Gly, 1.00; Phe, 1.10; Leu, 0.97.

Methods. Radioreceptor Binding Assay Using Rat Brain Membrane. Rat brain membrane was prepared as described by Waterfield et al.[42] The rediorecptor binding of enkephalins, β-endorphin, and naloxone was assayed by the method of Queen et al.[43] [^{3}H]Naloxone (100 μL, final concentration 2 nM) was added to the suspension of the rat brain membrane (0.8 mL) in 0.05 M Tris buffer (pH 7.4) containing bacitracin (50 μg/mL) in order to inhibit the degradation of enkephalins.[44] After incubation at 25 °C for 30 min, competing ligands (100 μL) containing bacitracin (50 μg/mL) were added to the suspension. It was then incubated at 25 °C for 30 min and centrifuged at 2000*g* for 20 min. To the pellet was added 2 N KOH (600 μL), and the suspension (500 μL) was pipetted out. The radioactivity was measured with a liquid scintillation counter. The concentration for 50% inhibition (IC_{50}) was determined by interpolating a logarithmic concentration vs. percent inhibition plot. Geometric means of IC_{50} values, estimated from the effects of at least ten concentrations with triplicate assays, were obtained.

Bioassays of Enkephalins in the Guinea Pig Ileum and the Mouse Vas Deferens. Two bioassay preparations differing in relative density of the various opiate receptors[45,46] were utilized. The guinea pig ileum[47] (GPI) was incubated at 36 °C in a modified

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Krebs solution of the following composition (mM): NaCl (118), KCl (4.75), KH_2PO_4 (1.19), $CaCl_2$ (2.54), $NaHCO_3$ (25), $MgSO_4$ (1.2), and glucose (11). The mouse vas deferens[48] (MVD) was incubated at 25 °C in a solution of the following composition (mM): NaCl (118), KCl (4.75), KH_2PO_4 (0.95), $CaCl_2$ (2.54), $NaHCO_3$ (25), glucose (11), tyrosine (0.25), and ascorbic acid (0.1). The bath solution was gassed with 95% O_2–5% CO_2. The tissue was stimulated at 0.1 Hz by current applied through a pair of platinum electrodes, and the electrically stimulated isometric contractions were recorded. [Leu^5]Enkephalin, DALE, or ENAPE was added to the bath. The IC_{50} was determined from regression analysis at several doses over and below 50% inhibition of the electrically stimulated twitch. Geometric means of IC_{50} values from several experiments were obtained.

Photolysis of Guinea Pig Ileum in the Presence of ENAPE. A GPI strip was put in a Pyrex organ bath and incubated in the same bath solution (5 mL) used in the bioassay experiment. ENAPE was added in a final concentration of either 10 or 100 nM. The preparation was photolyzed at room temperature with a Panasonic electronic flash (Model PE-3000) by direct exposure to the Xenon lamp after the plastic window was removed. The flash pulse was in the millisecond range, and the time interval of each flash was about 10 s. The strip was exposed to the flash at a distance of 5 cm. One hundred flashes were sufficient to photolyze the azido group of ENAPE completely. Then the bath solution was replaced several times with fresh solution. The response of the muscle strip to 90 nM [Leu^5]-enkephalin was recorded before and after photolysis. As a control, similar bioassay experiments were done under the same conditions with 5 and 20 nM previously photolyzed ENAPE. Photolysis of the GPI in the absence of ENAPE was also done as another control.

Effect of DALE on the Photolysis of Guinea Pig Ileum with ENAPE. After the addition of DALE (final concentration 20 μM) to the GPI strip in the Pyrex bath, the incubation mixture was subjected to 10 nM ENAPE and exposed to 100 flashes. The bath solution was replaced, and the electrically stimulated contractions were recorded. Higher doses of DALE and ENAPE (final concentrations 40 μM and 100 nM, respectively) were also studied. The response to 180 nM [Leu^5]enkephalin was recorded before and after photolysis as a measure of the muscle strip response.

Photolysis of Mouse Vas Deferens. Irradiations of MVD strips were done similarly to those of the GPI, except that the bath solution for the MVD bioassay was used. Doses of ENAPE and DALE were 10 nM and 10 μM, respectively.

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(Acylaryloxy)acetic Acid Diuretics. 5. [(2-Alkyl- and 2,2-Disubstituted-1,3-dioxo-5-indanyl)oxy]acetic Acids

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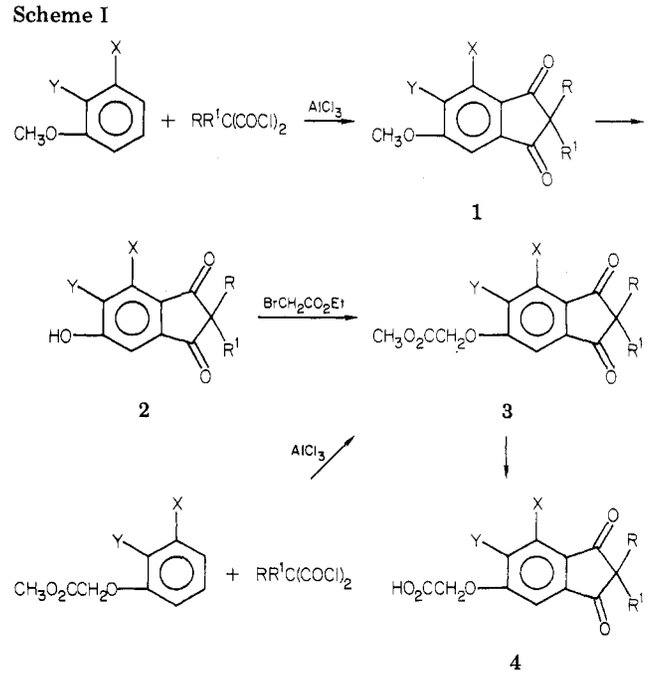
Investigation of the chemistry of the potent uricosuric diuretic indacrinone (MK-196) prompted the synthesis of a series of 3-oxo derivatives, i.e., the indan-1,3-diones. In general, both pharmacological parmameters (uricosuria and diuresis) were significantly less pronounced with the 1,3-diones than with the parent 1-oxo compounds.

In the first paper of this series[1] we reported on a number of [(2-alkyl- and 2,2-dialkyl-1-oxo-5-indanyl)oxy]acetic acids, which were shown to possess a high order of diuretic activity in rats, dogs, and chimpanzees and pronounced uricosuria in chimpanzees. Subsequent publications have dealt with the replacement of one of the 2-alkyl substituents with aryl moieties,[2] a series of 5-acylbenzofuran-2-carboxylic acids,[3] and a series of indeno[5,4-*b*]furans.[4] In this report, we describe the synthesis of [(1,3-dioxo-5-indanyl)oxy]acetic acids and the effects of this structural modification on saluretic and uricosuric activity.

Chemistry. Three synthetic routes to the indan-1,3-diones were employed. When 2,3-dimethylanisoles or 2-methyl-3-chloroanisoles or the corresponding oxyacetic acid esters were employed as starting materials, the Friedel–Crafts reaction with a suitably substituted malonyl chloride provided the indandiones. Ether cleavage of the anisoles, **1**, gave the phenolic derivatives, **2**, which were alkylated with bromoacetic acid ester and then hydrolyzed to the desired product, **4**. This sequence is shown in Scheme I.

Scheme I



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