basis was maintained by adjusting the concentration of the test compound in the diet weekly.

On day 15 all animals were necropsied. The stomachs were excised and weighed, and sections of each were fixed in neutral buffered 10% formalin for histological processing. The tissues were then cut, blocked, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

**Acknowledgment.** We thank the following individuals for providing skilled technical assistance: David J. Conn, Carol Gerhart, Razia K. Rizvi, and Glen Tetzloff. We are also deeply indebted to Drs. H. E. Black, E. H. Gold, B. F. Murphy, E. Schwartz, and R. J. Szot, for many stimulating and helpful discussions throughout the course of this work.

**Registry No.** 1, 76081-98-6; 2, 91849-04-6; 3, 79707-13-4; 4, 85333-23-9; 5, 91848-94-1; 6, 85333-50-2; 7, 85333-46-6; 8, 91848-84-9; 9, 85332-64-5; 10, 85332-81-6; 11, 79707-53-2; 12, 85333-09-1; 13, 110270-88-7; 14, 79707-49-6; 15, 45644-21-1; 16, 110270-89-8; 17, 110270-90-1; 18, 79707-22-5; 19, 79707-21-4; 20, 110294-56-9; 2,6-dichloropyridine, 2402-78-0; chloroacetone, 78-95-5; 2-amino-3-(phenylmethoxy)pyridine, 24016-03-3; methyl 3-bromo-2-oxobutyrate, 34329-73-2.

# Metabolic Synthesis of Arylacetic Acid Antiinflammatory Drugs from Arylhexenoic Acids. 2. Indomethacin

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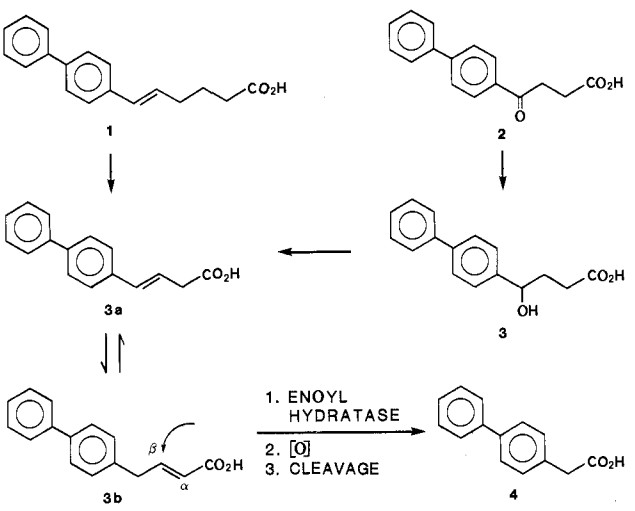
Arylacetic acid antiinflammatory drugs can be metabolically produced by β-oxidation of a 6-arylhex-5-enoic acid side chain. Such a mechanism provides for an in vivo sustained release of the active principle indomethacin from 6-[*N*-(*p*-chlorobenzoyl)-2-methylindol-3-yl]hex-5-enoic acid (**7**). Similarly, biphenylacetic acid was produced from both 6-(4′-biphenylyl)hex-5-enoic acid and its lower even homologue, 4-(4′-biphenylyl)but-3-enoic acid. The indole derivative produced sustained analgesia in a yeast-induced hyperalgesia model over a 12-h period. Indomethacin plasma levels of 2 μg/mL were observed for up to 24 h. Such levels were less than those achieved for the analogous case in which biphenylacetic acid was produced from biphenylylhex-5-enoic acid, suggesting metabolic discrimination between hex-5-enoic substrates. When indomethacin was dosed in equipotent analgesic levels, the level of circulating drug was considerably higher than that seen for metabolically derived drug. Hence 6-hex-5-enoic acid derivatives of indomethacin are metabolized to indomethacin in vivo to give sustained analgesia at low apparent circulating plasma levels of free drug. The possibility of tissue compartmentalization enhancing biological efficacy is suggested by these observations.

Toxicity resulting from acutely elevated drug concentrations following oral administration has provoked considerable research into drug delivery systems that facilitate zero-order kinetics in the absorption or distribution of the active principle. Numerous means are currently employed to achieve even drug distribution. They range from the relatively simple ester prodrug[1] stratagem to advanced pharmaceutical preparations that employ osmotic pumps, acting through polymeric membranes, to diffuse drug at a fixed rate via high-precision, laser-drilled holes.[2]

Metabolic production of a drug is one of the phenomena that can be exploited to unmask an inert prodrug and release its active form. Certain enzyme systems that are involved in ubiquitous metabolic processes can be exploited to effect systemic drug distribution. The most commonly used enzymes in prodrug design are the hydrolases, although oxidoreductases and lipases also serve. Furthermore, if distinct biochemical differences exist between pathological and normal cells, then selective toxicity may be achieved by either targeted drug delivery or site-specific activation.[3]

This paper will present evidence that indicates that arylhexenoic acids may act as arylacetic acid prodrugs, which in certain cases convey useful pharmacological characteristics to the parent drug. Initial evidence for such an assertion was found when *trans*-6-(4′-biphenylyl)hex-5-enoic acid (**1**) was found to have significant, long-duration, antiinflammatory and antiplatelet aggregatory properties in vivo[4] or ex vivo. The compound was essentially devoid of in vitro activity in appropriate models. It was found that the compound was undergoing metabolic activation in vivo by conversion to the known cyclooxygenase inhibitor biphenylacetic acid[5] (**4**), which was responsible for the observed biological activity. The metabolically produced drug profile, as determined by

**Scheme I**

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(5) See *Chem. Eng. News* **1967**, *45* (Feb 13), 10.

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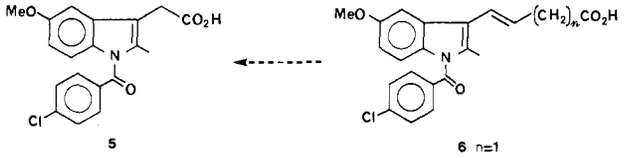
HPLC analysis of plasma, showed distinctly advantageous characteristics when compared to that derived from oral dosage. The peak level was dramatically reduced, and duration increased by a factor of twofold.

As shown in Scheme I, the conversion from a C-6 chain to a C-2 chain requires the equivalent of two successive β-oxidations.[6] The second is in fact the migration of the double bond in **3a** into conjugation with the carboxyl in **3b**. This may be an enzymic or chemical process. The compound is then in a suitable oxidation state for rapid β-hydration via a Michael-type addition of water catalyzed by enoyl hydratase. In fact, no evidence for the **3a** or **3b** form was found in plasma derived from either the *trans*-hexenoic acid **1** or its cis isomer. The rate-limiting step in β-oxidation is likely to be hydride abstraction; hence a preoxidized form, requiring only isomerization, is likely to be rapidly converted to its dinor derivative. This was observed to be the case when **3a** or its corresponding cis isomer was administered to several species (rat, guinea pig, and dog) and was found to be rapidly converted to biphenylacetic acid. The degradation of 4-(4′-biphenylyl)-4-oxobutanoic acid (Fenbufen) (**2**) to biphenylacetic acid may also be interpreted by a similar rationale. Reduction of the ketone may lead to a benzylic alcohol (**3**), which upon dehydration leads to the intermediate **3a** described above. The Michael adduct of **3b** and water, a racemate corresponding to the putative enoyl hydratase product, 4-(4′-biphenylyl)-3-hydroxybutanoic acid, is a known compound, BDH7538, and this compound has been shown in several species to be converted to biphenylacetic acid.[7] Prostanoid side chain degradation is reported to proceed through a similar mechanism.[8]

The facility with which the oxidative cleavage of biphenylacetic acid occurred in the biphenylylhex-5-enoic acid series suggested that such a modification might impart the same desirable characteristics of smooth, long-duration delivery of the active component to other antiinflammatory arylacetic acids.




5 6 n=1 7 n=3

Indomethacin (**5**) is a highly effective, nonsteroidal antiinflammatory drug whose characteristics have been modified by a variety of either prodrug or formulation formats in order to provide rate-limiting or zero-order drug delivery. Most chemical modifications have involved the formation of diverse esters of the indoleacetic acid.[1] It was of considerable interest therefore to examine C-3 chain extensions to indomethacin, an indoleacetic acid derivative, in the light of the above-mentioned observations for the biological conversions of biphenylylhexenoates, -butenoates, or -ketobutanoates. However, given the observed chemical instability of an indole amide linkage when an indole nucleus is acylated at position 3,[9] a precursor that would incorporate an acyl derivative, such as a 4-oxobutanoate or higher congener, was considered unsuitable, due to predictable formulation difficulties. On the other hand, substantially less chemical instability would be anticipated in *N*-(*p*-chlorobenzoyl) amides of 6-(5′-methoxy-2′-methylindol-3′-yl)hex-5-enoic acids (**7**) or their lower even congeners (**6**), and this was found to be the case. A potential limitation of this in vivo approach to an indoleacetic acid lies in the second step of the β-oxidation process, which requires a double-bond conjugation away from the indole nucleus, toward the carboxylic acid. The comparative difficulty of deconjugation for vinylindole systems relative to vinylbiphenyl systems may limit the directly analogous prediction of isomerization followed subsequently by oxidation and cleavage to an indoleacetic acid.

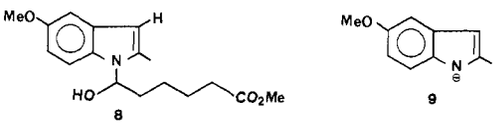
Other factors that may limit the generality of the β-oxidation process to produce indomethacin are the following. Altered metabolic sequences in the prodrug may cause premature elimination of the intact chain, e.g., demethylation or debenzoylation may become competitive with β-oxidation, and low levels of drug result. Altered pharmacokinetics, resulting from changes in physical characteristics of the prodrug, may result in a compartmentalization of prodrug that differs from that of the parent drug.

Both of these factors impinge on the development of a therapeutic agent from this prodrug concept, and although they add additional variables to control, they also permit additional customization of a drug's delivery system and its efficacy.

The indolylalkenoic acids **6** and **7** were synthesized and administered to two species, rats and guinea pigs. Biological assays for indomethacin-like activity (PBQ writhing and rat yeast paw edema) were carried out, in addition to plasma extractions and high-pressure liquid chromatographic (HPLC) analysis for both indomethacin and unchanged prodrug. In vitro tests showed that the intact prodrugs did not possess cyclooxygenase enzyme inhibitory properties.

## Results and Discussion

**Chemistry.** The synthetic sequence leading to the indolylalkenoic acid derivatives **6** and **7** is outlined in Scheme II. The acylation of indolylmagnesium bromide salts by acid anhydrides or acid chlorides is facilitated by the use of tetrahydrofuran as solvent, in which the salts are soluble, in contrast with diethyl ether. There is, however, an enhanced proportion of N-acylation in THF, reflecting the greater cation solvating powers of this solvent. Furthermore, when methyl 5-formylpentanoate was used as the alkylating agent, in THF, the exclusive product was *N*-(1-hydroxy-5-carbomethoxypentyl)-5-methoxy-2-methylindole (**8**), a quite stable indole hemiaminal. This selectivity of hydroxyalkylation may be suggestive of the differing hardness of the carbon vs. nitrogen anionic centers being unmasked by the dissociated ion pair in THF.[10] Aliphatic aldehydes and indolylmagnesium bromides react in ether to give 3-(hydroxyalkyl)indole derivatives.




8 9

The choice of an *N*-tosyl blocking group was pivotal to the successful completion of the synthesis. Thus, the

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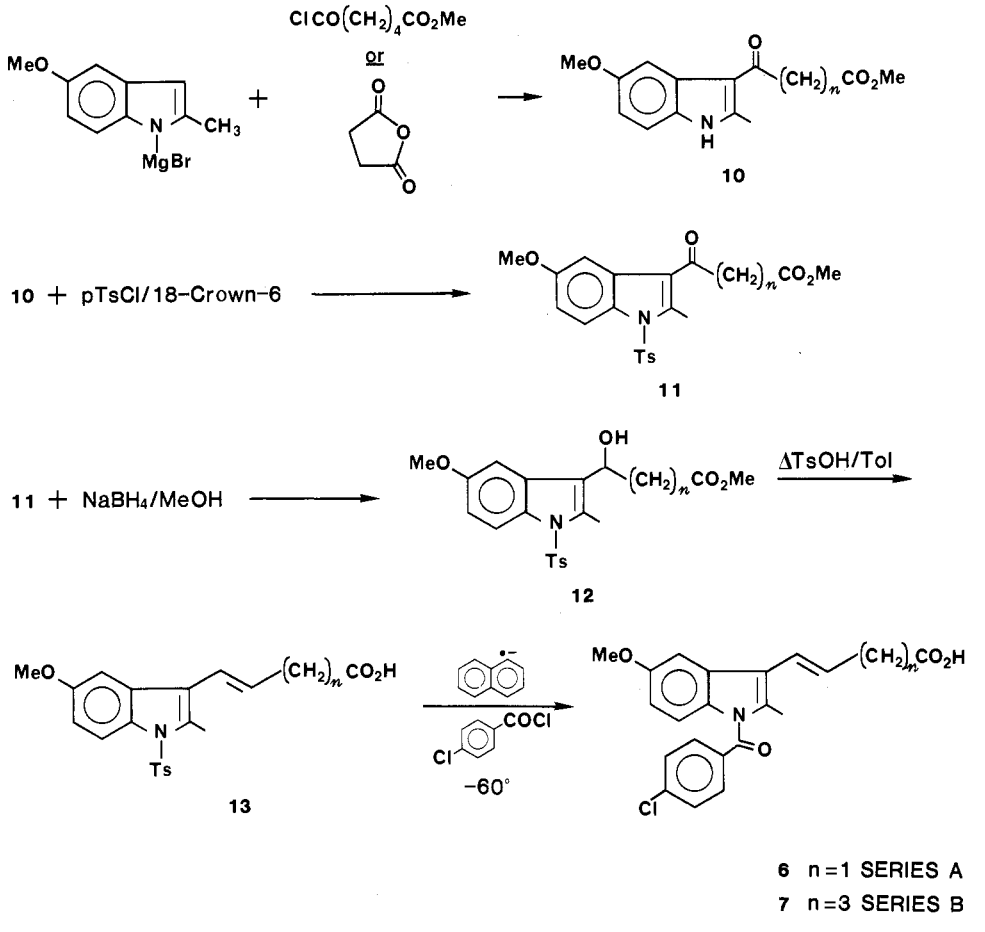
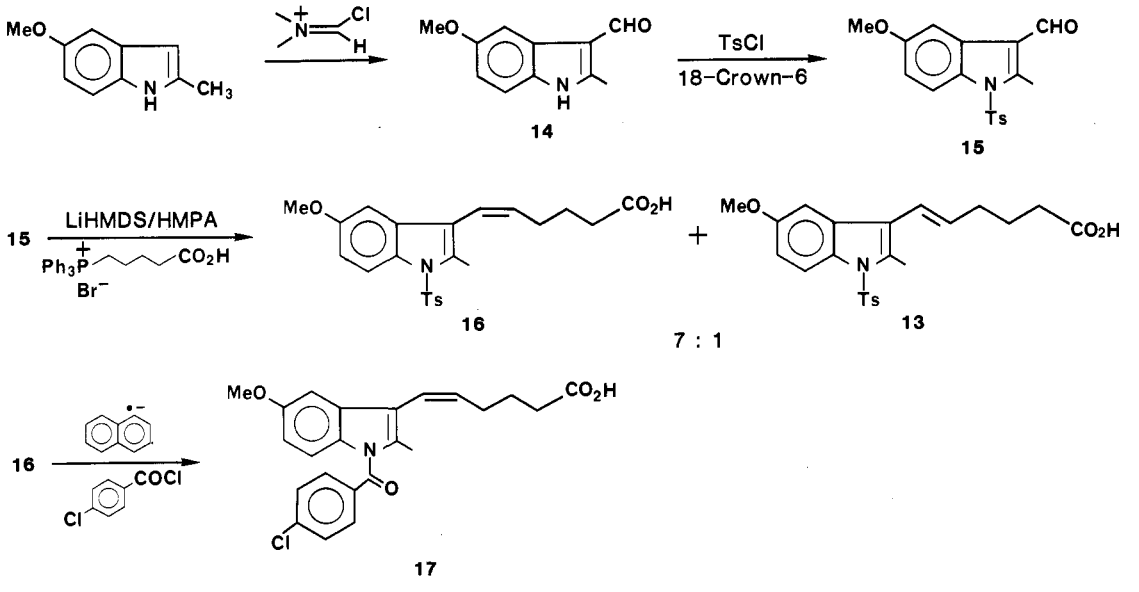
**Scheme II.** Series A and B




**Scheme III.** Series C




carbonyl functionality in 3-acylindoles shows very extensive delocalization, and in the case in question, **10**, the infrared spectrum shows an absorbance at 1600 $cm^{-1}$ and the compound cannot be reduced by borohydride reducing agents.[11] In contrast, the *N*-tosyl compound was quantitatively reduced by $NaBH_4$ in methanol to the stable alcohol **12**. Subsequent dehydration under acid-catalyzed conditions, followed by hydrolysis, led to the trans olefin **13** in high yield.

The second consequence of the choice of *N*-tosyl as a blocking group follows from the capability to selectively cleave sulfonamides under stoichiometric radical anion conditions, using sodium naphthalenide or sodium biphenylenide, at very low temperatures. This generally underexploited reaction[12] generated, in the above cases, the vinylindole N anion at −60 °C, which was selectively

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acylated with *p*-chlorobenzoyl chloride in situ, to yield the desired product **6** or **7** in a high-yield, one-pot sequence.

The synthesis of the requisite indole *N*-tosylate **11** was achieved either according to published procedures,[13] in which the indole 3-carbonyl derivative was treated with *p*-toluenesulfonyl chloride in 2-butanone in the presence of anhydrous $K_2CO_3$, or through a modification of this reaction, using crown ether catalysis with 18-crown-6. This latter method resulted in considerably shorter reaction times.

The synthesis of **17**, the cis analogue of **7**, was accomplished by the sequence outlined in Scheme III.

Vilsmeier formylation of 5-methoxy-2-methylindole leads directly to the substituted indole-3-carboxaldehyde. The solid-phase IR spectrum again reveals the extent of delocalization in such systems, since the "carbonyl" band appeared at 1600 $cm^{-1}$. However, in the proton NMR, a one-proton peak was present at $\delta$ 9.90, indicating that the aldehyde is not actually enolized. Indole-3-carboxaldehydes are unreactive to Wittig conditions.[11] The *N*-*p*-tosyl derivative **15**, however, behaved as a normal aldehyde. The *N*-tosylate **15**, formed from the $K_2CO_3$/18-crown-6 catalyzed condensation of *p*-toluenesulfonyl chloride and 5-methoxy-2-methylindole-3-carboxaldehyde (**14**), shows a carbonyl absorbance at 1665 $cm^{-1}$ and an aldehydic resonance at $\delta$ 10.20.

Compound **15** was then converted to a 7:1 cis/trans mixture of substituted indol-3-ylhex-5′-enoic acids **16** and **13** via a Wittig reaction in THF/HMPA, using hexamethyldisilazane/*n*-BuLi to generate the ylide of (5-carboxypentyl)triphenylphosphonium bromide. These isomers (**16** and **13**) were separated by HPLC after esterification with diazomethane and are individually characterized by the olefinic resonances at $\delta$ 6.35 (d, $J_{1,2}$ = 16 Hz) and 6.00 (d of t, $J_{2,1}$ = 16 Hz, $J_{2,3}$ = 4.5 Hz) for the trans compound and $\delta$ 6.24 (d, $J_{1,2}$ = 11 Hz) and 5.80 (d of t, $J_{2,1}$ = 11 Hz, $J_{2,3}$ = 6 Hz) for the cis compound. The indole 2-methyl group is shifted upfield by 0.22 ppm in the cis compared to the trans compound.

Hydrolysis to the free acid required for the final deblocking/acylation step was, however, accompanied by very substantial cis–trans isomerization, which is probably a consequence of the release of steric encumbrance from the 2-methyl substituent. Sufficient cis material was recovered by chromatography for conversion to the requisite *N*-(*p*-chlorobenzoyl)indole derivative in the manner described for the trans compound. Because of the tendency of the *N*-*p*-chlorobenzoyl derivative to undergo ready chemical isomerization to the trans form, no biological properties of the cis compound were determined.

**Pharmacology.** Inhibition of the writhing response induced by intraperitoneal administration of phenylbenzoquinone (PBQ) is known to be a sensitive assay for clinically useful antiinflammatory analgesic agents.[14] Indomethacin in this assay has a highly reproducible $ED_{50}$ of 3 mg/kg po, with a mean duration of 1 h following such a dosage. It thus serves as a convenient bioassay for the metabolic release of indomethacin from a prodrug format. The assay is, however, responsive to numerous other pharmacological mediators whose influences cannot be neglected if associated with the nonmetabolized form of the drug. Compound **7** at 4 h pretreatment significantly inhibited PBQ-induced writhing. Compound **6** was generally less active in this assay. The results are summarized in Table I.

Table I. Inhibition of Phenylbenzoquinone Writhing in Mice

| test substance | dose, mg/kg po | time, h | writhes (SE) | % inhibn[a] | no. |
|---|---|---|---|---|---|
| control (methanol) | | | 45.8 | | |
| indomethacin | 3 | 1 | 4.8 | 89 | 10 |
| | | 2 | 38.3 | 16 | 10 |
| | | 4 | 32.9 | 28 | 10 |
| **6** | 30 | 1 | 34.0 (4.0) | 19 | 20 |
| | | 2 | 28.6 (2.4) | 32 | 20 |
| | | 4 | 23.0 (4.1) | 46 | 20 |
| | | 6 | 29.5 (3.4) | 28 | 20 |
| **7** | 30 | 1 | 16.4 (2.2) | 20 | 10 |
| | | 2 | 15.3 (1.7) | 26 | 10 |
| | | 4 | 7.2 (2.7) | 65 | 10 |

[a] See Experimental Section for description.

Depression of the rat vocalization threshold in the yeast-inflamed rat paw is also particularly sensitive to antiinflammatory agents.[15] In this hyperalgesia assay, indomethacin has a highly reliable $ED_{50}$ of 5 mg/kg po at 1 h. The half-life for analgesia following a 10 mg/kg dose is approximately 3 h. The data for the indomethacin homologues are shown in Table II. Compound **7** is seen to have a considerably reduced acute potency in this assay compared to indomethacin, and such an observation is consistent with an inactive prodrug. However, as observed in the PBQ writhing assay, there is an onset of significant activity 4–6 h after dosing. The duration of analgesic activity following such a 30 mg/kg dose is also remarkably long, approximately 12 h. It is seen that the inflamed foot elicits a vocalization response indistinguishable from that of the normal control foot for approximately 8 h for indomethacin treatment at 10 mg/kg and 12 h for treatment with **7** at 30 mg/kg. Furthermore, over an 8-h period, **7**, when dosed at 10 mg/kg, produces significant analgesia, indistinguishable from the normal control at 4–8 h. In contrast, **6**, at 30 mg/kg, exhibited insignificant activity in this assay.

In order to correlate the observed in vivo results with a measure of serum drug concentration, blood was taken from animals following the hyperalgesia assay and serum was analyzed by HPLC for both indomethacin and the precursor. Table II shows that indomethacin was apparent throughout the 24-h period at a constant 2 $\mu$g/mL for compound **7**. Compound **6** was not metabolized to the same extent. The presence of indomethacin was shown by HPLC, at the quantitation limits of 600 ng/mL, or below. Thus, average plasma concentration of the C-4-derived indomethacin was one-fifth to one-tenth that derived from a C-6 alkenoic acid chain.

In indomethacin-treated rats ($n$ = 10 for each group) examined over a dose–response range of 0.30–10.0 mg/kg at 1 h pretreatment, excellent correlation was found between mean plasma levels of indomethacin and (a) mean vocalization thresholds ($r$ = 1.0), (b) mean percent analgesia ($r$ = 0.96), and (c) dosage ($r$ = 1.0). However, an examination of the data for **7** (Tables I and II) reveals significant and long-lasting analgesia (albeit resulting from a higher initial dosage) and inhibition of PBQ writhing, with the maximum effect appearing after an 8 h predose protocol. When attempts were made to correlate this observed activity in the rat with derived indomethacin levels in the plasma, no relationship was found. In fact, the observed plasma level of indomethacin at maximum analgesic activity, 2.1 ± 0.4 $\mu$g/mL, for prodrug **7** is lower

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**Table II.** Rat Yeast Induced Hyperalgesia Assay: Correlation with Selected Plasma Drug Levels

| test substance | dose | time, h | vocalization threshold,[a] mmHg ± (SEM)[b] normal | inflamed | % analgesic[c] | drug levels, μg/mL ± (SEM) indomethacin | drug 6 or 7 | *n* |
|---|---|---|---|---|---|---|---|---|
| control (Methocell) | | 1 | 25.8 (2.7) | 15.8 (0.9)*** | | | | 50 |
| | | 4 | 23.6 (3) | 14.4 (0.8)** | | | | |
| | | 8 | 25.4 (3.1) | 15.2 (0.8)** | | | | |
| indomethacin | 10 mg/kg | 1 | 27.9 (0.9) | 30.0 | 70 | 21.0 (2.8) | | 10 |
| | | 4 | 26.8 (1.9) | 32.0 (1.8)* | 100 | 38.0 (6.8) | | 10 |
| | | 8 | 28.4 (5.0) | 28.4 (5.0) | 40 | 21.0 (1.2) | | 10 |
| | | 12 | 24.4 (0.9) | 15.6 (1.4)*** | 0 | 9.8 (0.8) | | 10 |
| | | 24 | 22.4 (1.4) | 16.0 (2.1)** | 0 | 3.3 (0.3) | | 10 |
| **7** | 10 mg/kg | 1 | 24.0 (1.0) | 16.8 (1.0)*** | 10 | NT[d] | | 10 |
| | | 6 | 22.0 (1.7) | 21.6 (2.1) | 40 | | | 10 |
| | | 8 | 23.2 (0.8) | 23.8 (1.9) | 40 | | | 10 |
| **7** | 30 mg/kg | 1 | 23.0 (2.0) | 25.4 (1.7) | 60 | – | – | 10 |
| | | 4 | 24.8 (1.4) | 26.0 (2.1) | 60 | 2.2 (0.3) | 24.1 (2.1) | 5 |
| | | 8 | 25.6 (0.2) | 27.6 (3.9) | 100 | 2.1 (0.4) | 6.1 (2.1) | 5 |
| | | 12 | 25.2 (1.4) | 25.0 (2.8) | 60 | 2.2 | 1.1 (0.5) | 5 |
| | | 24 | 24.4 (1.6) | 13.0 (1.2)*** | 0 | 1.6 (0.5) | nil | 5 |
| control | | | 24.2 (1.7) | | | | | |
| **6** | 30 mg/kg | 1 | 25.8 (2.7) | 14.2 (3.0)** | 10 | <1 | 26.0 | 10 |
| | | 4 | 23.4 (2.9) | 20.2 (6.0) | 30 | <1 | 17.0 | 10 |
| | | 8 | 25.4 (2.1) | 21.6 (5.2) | 30 | <1 | 10.0 | 10 |

[a] Significance, paired *t* test between normal and inflamed foot: (***) $p < 0.001$, (**) $p < 0.05$, (**) $p < 0.1$; see Experimental Section for description. [b] Standard error of the mean. [c] Percent of group that had vocalization threshold >25 mmHg. [d] Not determined.

than the 24-h value for indomethacin free drug (3.3 ± 0.3 μg/mL) for which no analgesia was observed. At the lower dose of 10 mg/kg, analgesia increased over time to reach a maximum 8 h after dosing.

An explanation of this anomaly, keeping in mind the excellent correlations cited above for indomethacin, may be that differing tissue levels are involved and that the distribution coefficient of drug between tissue and plasma may be altered in drug resulting from metabolic sources compared to oral absorption. This would be the case if activation were to take place at the site of inflammation and the drug were sequestered in inflamed tissue. Acidic nonsteroidal antiinflammatory drugs have been shown to have significantly higher concentrations in inflamed tissue than in surrounding compartments.[16]

**Conclusion.** As evidenced by the identification of circulating indomethacin by HPLC, prodrugs that incorporate a hex-5-enoic acid or, to a much lesser extent, a but-3-enoic acid chain at the 3-position of the indole moiety are metabolized to the appropriately substituted indoleacetic acid, yielding a continuous and constant concentration over 24 h. The ratios of circulating prodrug to indomethacin in the species examined indicate, however, that the conversion is not efficient. If differential sequestration in inflamed tissue is not considered (an unexpected beneficial consequence), then as a prodrug strategy, metabolic activation by β-oxidation is not appropriate for indolylalkenoic acids. Indolylalkenoic acids appear to differ considerably from biphenylylalkenoic acids both in degree of production of acetic acids and in the relative efficiency of cleavage of C-4 to C-6 acids.

It should also be noted that in vivo read-out of activity following metabolic conversions is likely to be complicated by intrinsic activity of the prodrug. In this case, the cyclooxygenase inhibition characteristic of indomethacin and responsible for the action of most NSAID drugs was absent from either prodrug derivative.

Controlled release, by whatever mechanism, must be balanced by controlled clearance in order for the accumulated steady-state concentration to become high enough to produce an effect. In animal models, where both mechanisms are metabolic, meaningful translations to the human system become doubly difficult. Firstly, it has been shown in the case of BDH 7538 and Fenbufen that the rate of conversion of the arylalkanoic acid chain to an arylacetic acid is much slower in humans than in other species.[17] Secondly, metabolism of indomethacin is species-specific, fairly rapid hydrolysis of the indole amide bond being the principal mode of metabolism in guinea pigs and rats, whereas in humans, clearance of the intact drug is the mode of excretion. Both of these factors, metabolic production and metabolic clearance, would be anticipated to affect any potential application in a human system, and as neither can be assessed a priori, the introduction of metabolic activation as a prodrug strategy requires new standards of correlation between species.

## Experimental Section

Melting points were taken in capillary tubes and are uncorrected. Elemental analysis (C, H, N, Cl, S) were performed by Guelph Chemical Laboratories, Guelph, Ontario, and are in agreement with calculated values ±0.4%. $^1$H NMR spectra were recorded on a Varian 390 90-MHz spectrometer using TMS as internal standard. Sprague–Dawley rats were supplied by Canadian Breeding Laboratories. CD-1 mice were supplied by Charles River Laboratories, Kingston, NY.

**Series A. 3-(3′-Carbomethoxypropanoyl)-5-methoxy-2-methylindole (10).** A solution of 30 g of 5-methoxy-2-methylindole (0.18 mol) dissolved in 300 mL of dry THF, under $N_2$, was cooled to 0 °C and treated with 46 mL of 3 M MeMgBr in THF. After evolution of methane at room temperature for 30 min, the solution was cooled to 0 °C and succinic anhydride (0.17 mol) in 75 mL of THF was added. The reaction mixture was warmed to room temperature for 45 min and cooled to 0 °C, whereupon it was quenched with 20% citric acid (20 mL). The organic phase, diluted with 200 mL of ether, was separated, dried, and concentrated. The *N*-carboxypropanoyl derivative accompanying the desired product was hydrolyzed in methanol (50 mL) and 1 N KOH (20 mL). Acidification with 20% citric acid to pH 5, followed by extraction with $CH_2Cl_2$, yielded 19 g (0.06 mol) of pure acid after crystallization from EtOAc. A further 6 g was recovered from the liquors (58%); mp 192–194 °C dec. The acid was esterified by refluxing 17 g (0.053 mol) in 500 mL of methanol containing 10 mL of $BF_3$·$OEt_2$ for 25 min. Solid $NaHCO_3$ (15 g) was added, and the solution was filtered and concentrated to deposit 3-(3′-carbomethoxypropanoyl)-5-methoxy-2-methylindole

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(16 g, 90% yield): mp 149–151 °C; $^1$H NMR ($CDCl_3$) δ 7.60 (d, $J_{4,6}$ = 3 Hz, H-4), 7.38 (d, $J_{7,6}$ = 9 Hz, H-7), 6.94 (dd, $J_{6,4}$ = 9 Hz), 4.00 (O*Me*), 3.90 ($CO_2$*Me*), 3.35, 2.95 (m, ($CH_2$)$_2$$CO_2$Me), 2.74 (s, indole $CH_3$), 9.7 (N*H*).

Anal. ($C_{15}H_{17}NO_4$) C, H, N.

***N*-(*p*-Tolylsulfonyl)-3-(3′-carbomethoxypropanoyl)-5-methoxy-2-methylindole (11).** A solution of 5 g of 3-(3′-carbomethoxypropanoyl)-5-methoxy-2-methylindole (0.016 mol) in 100 mL of 2-butanone was treated with *p*-toluenesulfonyl chloride (TsCl) (6.80 g, 0.033 mol) and $K_2CO_3$ (10.1 g, 0.072 mol). The reaction mixture was refluxed for 3 h, filtered, concentrated, washed with hexane to remove excess TsCl, and chromatographed on silica gel to yield 3.8 g (54%) of the desired product after recrystallization from methanol: mp 131–132 °C; $^1$H NMR ($CDCl_3$) δ 8.15 (d, $J_{7,6}$ = 9 Hz, H-7), 7.65, 7.20 (d, *J* = 9 Hz, tosyl aromatic), 7.40 (d, $J_{4,6}$ = 3 Hz, H-4), 6.95 (dd, $J_{6,4}$ = 3 Hz, $J_{6,7}$ = 9 Hz, H-6), 3.90 (s, O*Me*), 3.70 (s, $CO_2$*Me*), 2.90 (s, indole *Me*), 2.40 (s, tosyl *Me*), 3.20, 2.80 (2 m, ($CH_2$)$_2$$CO_2$Me).

Anal. ($C_{22}H_{23}NO_6S$) C, H, N, S.

***N*-(*p*-Tolylsulfonyl)-3-(3′-carbomethoxy-1′-hydroxypropyl)-5-methoxy-2-methylindole (12).** To a solution of 6 g of *N*-(*p*-tolylsulfonyl)-3-(3′-carbomethoxypropanoyl)-5-methoxy-2-methylindole (0.014 mol) dissolved in MeOH/THF (1:1) (400 mL) and cooled to 0 °C was added 4 g of $NaBH_4$ (0.11 mol) over 1.5 h in 0.50-g portions. The reaction was complete after 1.5 h. Citric acid (20%) (200 mL) was added, and the solution was extracted with $CH_2Cl_2$ (5 × 50 mL). The concentrated, dried crude product was chromatographed on silica gel (30:70 EtOAc/hexane) to yield 4.9 g of product (82.6%): mp 83–84 °C; $^1$H NMR ($CDCl_3$) δ 7.97 (d, $J_{7,6}$ = 9 Hz, H-7), 7.50, 7.10 (d, *J* = 8 Hz, tosyl aromatic), 7.10 (d, $J_{4,6}$ = 3 Hz, H-4), 4.90 (t, $J_{4',3'}$ = 5 Hz, C*H*(OH)), 3.68 (s, O*Me*), 3.50 (s, $CO_2$*Me*), 2.40 (s, indole *Me*), 2.20 (s, tosyl *Me*), 2.10, 1.70 (2 m, ($CH_2$)$_2$$CO_2$Me).

Anal. ($C_{22}H_{25}NO_6S$) C, H, N, S.

***N*-(*p*-Tolylsulfonyl)-3-(3′-carbomethoxyprop-1′-enyl)-5-methoxy-2-methylindole (13a Methyl Ester).** To a solution of 4 g of *N*-(*p*-tolylsulfonyl)-3-(3′-carbomethoxy-1′-hydroxypropyl)-5-methoxy-2-methylindole (0.009 mol) in toluene (400 mL) under $N_2$ was added TsOH·$H_2O$ (450 mg, 0.003 mol). The reaction mixture was heated at 75 °C for 4 h, after which time the starting material and intermediate products had converted to the final product. The toluene solution was washed with $NaHCO_3$ (aqueous) (5 × 30 mL) and water, dried ($Na_2SO_4$), and concentrated to yield 3.4 g of product (89.2%) after crystallization with methanol as solvent: mp 100–101 °C; $^1$H NMR ($CDCl_3$) δ 8.20 (d, $J_{7,6}$ = 8 Hz, H-7), 7.20, 7.66 (d, *J* = 7 Hz, tosyl aromatic), 7.30 (d, $J_{4,6}$ = 3 Hz, H-4), 7.00 (dd, $J_{6,4}$ = 3 Hz, $J_{6,7}$ = 8 Hz, H-6), 6.50 (d, $J_{4,3}$ = 17 Hz, H-4′), 6.20 (dt, $J_{3',4'}$ = 17 Hz, $J_{3',2'}$ = 5.5 Hz, H-5), 3.90 (s, O*Me*), 3.78 (s, $CO_2$*Me*), 3.35 (d, $J_{2',3'}$ = 5.5 Hz, H-2′), 2.64 (s, indole *Me*), 2.40 (s, tosyl *Me*).

Anal. ($C_{22}H_{23}NO_5S$) C, H, N, S.

***N*-(*p*-Tolylsulfonyl)-3-(3′-carboxyprop-1′-enyl)-5-methoxy-2-methylindole (13a).** A solution of 3.4 g of *N*-(*p*-tolylsulfonyl)-3-(3′-carbomethoxyprop-1′-enyl)-5-methoxy-2-methylindole (0.008 mol) dissolved in 30 mL of THF/MeOH (1:1) was treated with 1 N KOH (4 equiv = 38.1 mL). The solution was heated to 40 °C for 3 h, neutralized with citric acid (20%), and extracted with $CH_2Cl_2$ (5 × 100 mL). Chromatography on silica gel (5% MeOH/$CH_2Cl_2$) yielded 3.00 g of product (94.5%): mp 185–186 °C.

Anal. ($C_{21}H_{21}NO_5S$) C, H, N, S.

***N*-(*p*-Chlorobenzoyl)-3-(3′-carboxyprop-1′-enyl)-5-methoxy-2-methylindole (6).** A solution of sodium naphthalenide was prepared from 570 mg of Na (24 mmol) in dimethoxyethane (DME) to which was added naphthalene (3.07 g) (24.0 mmol). The anion radical was allowed to form at room temperature for 2 h. The naphthalenide solution was cooled to −60 °C, and a solution of 3 g of indole tosylate **13a** (0.008 mol) in 90 mL of DME was added over 1 h. The reaction mixture was allowed to come to −40 °C for 1 h and recooled to −60 °C, and a solution of 2.04 g of *p*-chlorobenzoyl chloride (0.012 mol) in 20 mL of DME was added. The reaction mixture was allowed to come to room temperature for 1.0 h and cooled to 0 °C, and 50 mL of ice-cold citric acid (20% w/v) was added. The solution was extracted with chloroform (3 × 60 mL), and the organic phase was washed ($H_2O$), concentrated, and chromatographed on silica gel ($CH_2Cl_2$, 10% MeOH) to yield 2.6 g of **6** (84.7% yield): $^1$H NMR ($CDCl_3$) δ 7.65, 7.55 (dd, *p*-chlorobenzoyl), 7.34 (d, $J_{4,6}$ = 2 Hz, H-4), 7.10 (d, $J_{7,6}$ = 9 Hz, H-6), 6.70 (d, $J_{4',2'}$ = 17 Hz, H-4′), 6.34 (dt, $J_{3',4'}$ = 17 Hz, $J_{3',2'}$ = 5 Hz, H-3′), 3.90 (s, O*Me*), 3.20 (d, $J_{2',3'}$ = 5 Hz, H-2′), 2.42 (s, indole *Me*).

Anal. ($C_{21}H_{18}NO_4Cl$) C, H, N, Cl.

**Series B. 3-(5′-Carbomethoxypentanoyl)-5-methoxy-2-methylindole (10).** A sample of 20 g of 5-methoxy-2-methylindole (0.124 mol) was dissolved in dry THF (230 mL) at 0 °C, and 40 mL of 3 M MeMgBr was added. The reaction mixture was maintained at 30–40 °C until all methane had evolved. The reaction mixture was cooled to 0 °C, and a solution of 20 g of methyl 5-(chloroformyl)pentanoate (0.122 mol) in 20 mL of THF was added. The reaction mixture was stirred at 10 °C for 3 h. Citric acid (20%, aqueous) was added (50 mL), and the organic phase was separated after addition of 400 mL of ether, concentrated, taken up in $CH_2Cl_2$, dried ($Na_2SO_4$), and concentrated, to yield 19 g of a mixture of products, from which the desired product **10** was crystallized with methanol (12.9 g, 34.8% yield): mp 153–153.5 °C; $^1$H NMR ($CDCl_3$) δ 7.55 (d, $J_{4,6}$ = 3 Hz, H-4), 7.20 (d, $J_{7,6}$ = 8 Hz, H-7), 6.77 (dd, $J_{6,7}$ = 8 Hz, $J_{6,4}$ = 3.0 Hz, H-6), 3.82 (s, O*Me*), 3.60 (s, $CO_2$*Me*), 3.37 (s, N*H*), 2.60 (s, $CH_3$), 2.82, 2.35, 1.80 (3 m, CO($CH_2$)$_4$$CO_2$Me).

Anal. ($C_{17}H_{21}NO_4$) C, H, N.

Additional *N*-(5′-carbomethoxypentanoyl)-5-methoxy-2-methylindole (3 g) and some bisacylated product, 3-(5′-carbomethoxypentanoyl)-*N*-(5′-carbomethoxypentanoyl)-5-methoxy-2-methylindole (1.6 g), were recovered from the mother liquors following chromatography on silica gel with 10% EtOAc in hexane.

**3-(5′-Carbomethoxypentanoyl)-*N*-(5′-carbomethoxypentanoyl)-5-methoxy-2-methylindole:** isolated from the above chromatography; mp 72–73 °C.

Anal. ($C_{24}H_{31}NO_7$) C, H, N.

***N*-(*p*-Tolylsulfonyl)-3-(5′-carbomethoxypentanoyl)-5-methoxy-2-methylindole (11).** To a solution of 46 g of 3-(5′-carbomethoxypentanoyl)-5-methoxy-2-methylindole (0.152 mol) dissolved in $CH_2Cl_2$ (3 L) was added 72 g (0.520 mol) of anhydrous $K_2CO_3$, 32 g of *p*-toluenesulfonyl chloride (0.168 mol) and 18-crown-6 (430 mg). The reaction mixture was refluxed under $N_2$ for 20 h, filtered, and washed with $NaHCO_3$ (aqueous). Concentration to 500 mL followed by successive addition of methanol with concentration to remove $CH_2Cl_2$ resulted in crystallization of 36 g of the desired product. The mother liquors were chromatographed to produce an additional 3.6 g (silica gel, 10% EtOAc/hexane) (59.2% yield): mp 121–122 °C; $^1$H NMR ($CDCl_3$) δ 8.10, 7.16 (d, *J* = 9 Hz, tosyl), 7.57 (d, $J_{7,6}$ = 9 Hz, H-7), 7.27 (d, $J_{4,6}$ = 3 Hz, H-4), 6.80 (dd, $J_{6,7}$ = 9 Hz, H-6), 3.80 (s, O*Me*), 3.60 (s, $CO_2$Me), 2.75 (s, indole $CH_3$), 2.25 (s, tosyl $CH_3$), 2.85, 2.30, 1.65 (m, CO($CH_2$)$_4$$CO_2$Me).

Anal. ($C_{24}H_{27}NO_6S$) C, H, N, S.

***N*-(*p*-Tolylsulfonyl)-3-(5′-carbomethoxy-1′-hydroxypentyl)-5-methoxy-2-methylindole (12).** To a solution of 3.8 g of *N*-(*p*-tolylsulfonyl)-3-(5′-carbomethoxypentanoyl)-5-methoxy-2-methylindole (0.0086 mol) dissolved in 400 mL of MeOH/THF (1:1) was added $NaBH_4$ in 2 × 500 mg lots over 1 h. The solution was concentrated, $CH_2Cl_2$ was added (300 mL), and the solution was washed with $NH_4Cl$ solution (saturated) and $H_2O$ and dried ($Na_2SO_4$). Concentration with azeotropic removal of water gave the desired product (3.4 g) (89.5% as an oil): $^1$H NMR ($CDCl_3$) δ 8.08, 7.05 (d, *J* = 9 Hz, tosyl), 7.55 (d, $J_{7,6}$ = 9 Hz, H-7), 7.15 (d, $J_{4,6}$ = 3 Hz, H-4), 6.80 (dd, $J_{6,4}$ = 3 Hz, $J_{6,7}$ = 9 Hz, H-6), 4.84 (t, *J* = 5 Hz, $CH_2CH$(OH)), 3.78 (s, CO*Me*), 3.58 (s, $CO_2$*Me*), 2.47 (s, indole $CH_3$), 2.28 (s, tosyl $CH_3$), 2.20, 1.80, 1.40 (3 m, CH(OH)($CH_2$)$_4$$CO_2$Me).

Anal. ($C_{24}H_{29}NO_6S$) C, H, N, S.

***N*-(*p*-Tolylsulfonyl)-3-(5′-carbomethoxypent-1′-enyl)-5-methoxy-2-methylindole (13b Methyl Ester).** To 3.4 g of *N*-(*p*-tolylsulfonyl)-3-(5′-carbomethoxy-1′-hydroxypentyl)-5-methoxy-2-methylindole (12) (0.0076 mol) in 400 mL of toluene (under $N_2$) was added 300 mg of *p*-toluenesulfonic acid. The solution was heated to 65 °C for 0.5 h. The solution was extracted with 2 × 100 mL of $NaHCO_3$ (5%), washed with water (2 × 50 mL), dried, and concentrated to yield 3.2 g (98.2%) of crystalline product: mp 74–75 °C; $^1$H NMR ($CDCl_3$) δ 8.00, 7.00 (d, *J* = 9 Hz, tosyl), 7.58 (d, $J_{7,6}$ = 9 Hz, H-7), 6.90 (d, $J_{4,6}$ = 3 Hz, H-4), 6.72 (dd, $J_{6,7}$ = 9 Hz, $J_{6,4}$ = 3 Hz), 6.20 (d, $J_{6',5'}$ = 17 Hz, H-6′),

5.82 (dt, $J_{5',6'}$ = 17 Hz, $J_{5',4'}$ = 6 Hz, H-5'), 3.65 (s, OMe), 2.40 (s, indole $CH_3$), 2.13 (s, tosyl $CH_3$), 2.10, 1.70 (2 m, CH=CH-$(CH_2)_3CO_2H$).

Anal. ($C_{24}H_{27}NO_5S$) C, H, N, S.

***N*-(*p*-Tolylsulfonyl)-3-(5'-carboxypent-1'-enyl)-5-methoxy-2-methylindole (13b).** A solution of 2.0 g of *N*-(*p*-tolylsulfonyl)-3-(5'-carbomethoxypent-1'-enyl)-5-methoxy-2-methylindole (0.0047 mol) in 200 mL of MeOH was treated with 10 mL of 1 N KOH. The solution was refluxed for 35 min and left to stand for 16 h. Citric acid was added (35 mL) and the solution concentrated. $CH_2Cl_2$ was added and the organic phase separated, dried, and concentrated to yield **13b** (1.97 g) as an amorphous solid (96.0% yield): mp 117–119 °C.

Anal. ($C_{23}H_{25}NO_5S$) C, H, N, S.

***N*-(*p*-Chlorobenzoyl)-3-(5'-carboxypent-1'-enyl)-5-methoxy-2-methylindole (7).** A solution of sodium naphthalenide (4.5 mmol) (from 105 mg of Na in 18 mL of DME and 580 mg of naphthalene) was prepared at room temperature with vigorous stirring for 1 h.

The deep-green solution was cooled to −60 °C (at −78 °C the salt precipitates). A solution of 850 mg of *N*-(*p*-tolylsulfonyl)-3-(5'-carboxypent-1'-enyl)-5-methoxy-2-methylindole (2 mmol) in 19 mL of DME was added dropwise (all color disappears upon completion). The reaction was allowed to proceed at −40 °C for 1 h. To this solution was added 350 mg of *p*-chlorobenzoyl chloride (2 mmol) in 3 mL of DME. The reaction mixture was allowed to come to room temperature, the solution was treated with 35 mL of citric acid solution (20%), $CH_2Cl_2$ (100 mL) was added, and the organic phase was separated, dried ($Na_2SO_4$), concentrated, and chromatographed on silica gel (5% MeOH/$CH_2Cl_2$) to yield 593 mg of **7** (72% yield): mp 80–81 °C; $^1$H NMR ($CDCl_3$) δ 7.60, 7.40 (d, *J* = 7 Hz, *p*-chlorobenzoyl), 7.21 (d, $J_{4,6}$ = 3 Hz, H-4), 7.08 (d, $J_{7,6}$ = 8 Hz, H-7), 6.74 (dd, $J_{6,4}$ = 8 Hz, H-6), 6.51 (d, $J_{6',5'}$ = 17 Hz, H-6'), 6.12 (dt, $J_{5',6'}$ = 17 Hz, $J_{5',4'}$ = 6 Hz, H-5'), 3.87 (s, O*Me*), 2.40 (s, indole $CH_3$), 2.30, 1.90 (m, $(CH_2)_3CO_2H$).

Anal. ($C_{23}H_{22}NO_4Cl$) C, H, N, Cl.

**Series C. 3-Formyl-5-methoxy-2-methylindole (14).** To 109.5 g of *N*,*N*-dimethylformamide (DMF) (1.50 mol), cooled to −5 °C, was added 46 g of $POCl_3$ (0.300 mol) dropwise with stirring. After 10 min, 24 g of 5-methoxy-2-methylindole (0.150 mol) in 100 mL of DMF was added and the temperature was allowed to rise to 20 °C for 1 h. The reaction mixture was poured onto 1 L of 1 N NaOH in ice and stirred vigorously. The crystalline product was collected, washed with $H_2O$, and dried in vacuo to yield 22 g of **14** (76% yield): mp 199 °C (lit.[18] 198 mp °C).

***N*-(*p*-Tolylsulfonyl)-3-formyl-5-methoxy-2-methylindole (15).** To a solution of 4 g of 3-formyl-5-methoxy-2-methylindole (0.021 mol), 6 g of $K_2CO_3$ (0.043 mol), and 3.8 g of *p*-toluenesulfonyl chloride (0.021 mol) in 600 mL of $CH_2Cl_2$ was added 18-crown-6 (400 mg). The reaction mixture was refluxed for 24 h, after which the majority of starting material was consumed. The reaction mixture was filtered, washed with $NaHCO_3$ (aqueous 5%), concentrated, and chromatographed on silica gel (15% EtOAc/hexane) to yield 4 g of **15** (56% yield): mp 188–189 °C; $^1$H NMR ($CDCl_3$) δ 10.28 (s, CHO), 8.10 (d, $J_{7,6}$ = 9 Hz, H-7), 7.77, 7.28 (d, *J* = 9 Hz, tosyl), 7.72 (d, $J_{4,6}$ = 3 Hz, H-4), 7.70 (d, $J_{7,6}$ = 9 Hz, H-7), 7.00 (dd, $J_{6,7}$ = 9 Hz, $J_{6,4}$ = 3 Hz, H-6), 3.85 (s, OMe), 2.90 (s, indole $CH_3$), 2.30 (s, tosyl $CH_3$).

Anal. ($C_{18}H_{17}NO_4S$) C, H, N, S.

***N*-(*p*-tolylsulfonyl)-3-(5'-carbomethoxypent-1'-enyl)-5-methoxy-2-methylindole (Cis and Trans) (16 and 13b Methyl Esters).** A solution of 1.38 g (0.008 mol) of hexamethyldisilazane in THF (5 mL) was treated with 5.0 mL of an *n*-BuLi 1.6 M solution in hexane (0.008 mol) at 0 °C. The solution was diluted with HMPA (4 mL) and added to a suspension of 1.80 g of (5-carboxypentyl)triphenylphosphonium bromide (0.004 mol) in THF/HMPA (16 mL, 1:1 solution). The solution was stirred at 20 °C for 30 min, cooled to −78 °C, and added dropwise, with stirring, over 20 min, to a solution of 1.28 g of *N*-(*p*-tolylsulfonyl)-3-formyl-5-methoxy-2-methylindole (0.004 mol) in 12 mL of THF/HMPA (1:1). The reaction mixture was allowed to warm to room temperature, stirred overnight, and poured into citric acid (20% w/v). Ether was added (100 mL) and the organic phase separated, washed with water, and treated with excess diazomethane. The products (800 mg, 40.3% yield) separated by HPLC (Waters Prep 500, silica gel cartridge, using 10% ethyl acetate in hexane as solvent, with recycling unit the peaks resolved) were found to be 90 mg of trans **13** methyl ester, spectroscopically identical with **13** methyl ester derived from series B, and 710 mg of cis **16** methyl ester (oil). These compounds were distinguished from each other by the coupling constants of the olefinic protons. $^1$H NMR: δ 6.24 (d, $J_{1',2'}$ = 11 Hz), 5.80 (dt, $J_{2',1'}$ = 11 Hz, $J_{2',3'}$ = 6 Hz), 3.85 (s, $CO_2Me$), 3.52 (s, OMe), 2.39 (s, indole 2-$CH_3$), 2.30 (s, tosyl $CH_3$), 2.20 (m, s, hexenoic acid $(CH_2)_3$).

**Inhibition of Phenylbenzoquinone (PBQ) Writhing in Mice.** Groups of 10 male mice ($CD_1$, 18–22 g) were food-deprived overnight prior to the experiments. Test substances, suspended or dissolved in 1% methylcellulose, were administered orally (0.1 mL/10 g body weight) at various times prior to administration of PBQ (2.0 mg/kg ip). The mice were placed in individual boxes and observed for 10 min (5–15 min after PBQ). The number of "writhes" (abdominal contraction, lordosis, and hindlimb extension) for each animal was recorded, and the group means and standard errors were calculated. The means obtained from drug-treated groups were compared with the vehicle control mean values, and percent inhibition of writhing was calculated as follows: 100 − [(drug group mean/control group mean) × 100].

**Rat Yeast Induced Hyperalgesia Assay.** Groups of 10 female rats (Sprague–Dawley, 50–60 g) were food-deprived overnight prior to the experiments. Test substances were suspended or dissolved in 1% methylcellulose for oral administration (in 0.1 mL/10 g body weight) 2 h after a subplantar injection of brewer's yeast (5% in 0.1 mL). Vocalization thresholds were determined by applying pressure to the plantar surface of the normal (noninjected) and inflamed (yeast-injected, hyperalgesic) paw by means of an air-driven piston (diameter 2 mm). The pressure required to elicit a vocalization response was monitored by a manometer inserted into the air line. In 50 vehicle-treated control groups, the vocalization thresholds (±SEM) were found to be 25.8 ± 2.7 mmHg for the normal foot and 15.8 ± 0.9 mmHg for the hyperalgesic foot. These means were significantly different from each other at all time points in the experiment. Antiinflammatory agents have been reported to affect only the hyperalgesic foot vocalization threshold, and in the present studies, an animal was considered to be "analgesic" if the pressure required to elicit vocalization was equal to or greater than 25 mmHg for the hyperalgesic foot. Vocalization pressure for the normal foot was also recorded for significance determinations. Significance was measured by a paired *t* test comparison between the vocalization thresholds of the normal foot and the inflamed foot within each group of animals. Animals with no significant difference between normal and control values were considered to be analgesic. For duration studies, each group of animals was tested only once, at various times after oral drug administration but always 3 h after the subplantar injection of brewer's yeast. The 1-h test therefore represents the only nonprophylactic dose in the protocol.

**Plasma Analysis.** Plasma was submitted to high-performance liquid chromatography analysis for determination of the test compound and of its potential metabolite, indomethacin. The sample preparation was carried out by mixing 50 μL of plasma with an equal volume of 0.1 N hydrochloric acid, and the resulting mixture (10–20 μL) was injected directly onto a reverse-phase C-18 μBondapak column (3.9 mm × 30 cm, Waters Scientific Ltd.). The detection was performed with a UV detector set at 254 nm. Elution was carried out at a flow rate of 1.5 mL/min, with a mixture of methanol/water (65:35 v/v). The aqueous phase was a 0.01 M phosphate buffer adjusted to pH 4 by addition of dilute sodium hydroxide. Under these conditions, indomethacin eluted at approximately 10 min and the test compounds at approximately 14 min. The concentration of test compounds and indomethacin was determined from calibration using standard solutions in the usual manner.

**Acknowledgment.** We thank A. Rackham and A. Dallob of Merck Sharp & Dohme Research Laboratories for the determination of the biological results. We are grateful to Dr. J. G. Atkinson for helpful discussions in the preparation of the manuscript.

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**Registry No.** 5, 53-86-1; 6, 109908-76-1; 7, 109908-77-2; 10 ($n$ = 2), 109908-78-3; 10 ($n$ = 4), 109908-83-0; 10 (acid, $n$ = 2), 109908-92-1; 11 ($n$ = 2), 109908-79-4; 11 ($n$ = 4), 109908-85-2; 12 ($n$ = 2), 109908-80-7; 12 ($n$ = 4), 109908-86-3; 13a, 109908-82-9; 13a methyl ester, 109908-81-8; 13b, 109908-88-5; 13b methyl ester, 109908-87-4; 14, 6260-86-2; 15, 109908-89-6; 16, 109908-90-9; 17, 109908-91-0; TsCl, 98-59-9; 5-methoxy-2-methylindole, 1076-74-0; succinic anhydride, 108-30-5; p-chlorobenzoyl chloride, 122-01-0; 5-(chloroformyl)pentanoate, 102939-46-8; 3-(5'-carbomethoxypentanoyl)-N-(5'-carbomethoxypentanoyl)-5-methoxy-2-methylindole, 109908-84-1; (5-carboxypentyl)triphenylphosphonium bromide, 50889-29-7.

# Synthesis and Physicochemical Properties of Thiadiazolo[3,2-*a*]pyrimidinesulfonamides and Thiadiazolo[3,2-*a*]triazinesulfonamides as Candidates for Topically Effective Carbonic Anhydrase Inhibitors

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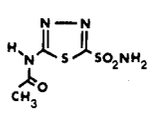
A series of bicyclic 1,3,4-thiadiazolo[3,2-*a*]pyrimidine- and 1,3,4-thiadiazolo[3,2-*a*]triazine-7-sulfonamides were synthesized from 5-amino-1,3,4-thiadiazole-2-sulfonamide and evaluated for topical efficacy as ocular hypotensive agents. The compounds were tested for the physicochemical properties of sulfonamide p$K_a$, free acid water solubility, $CHCl_3$/buffer partition, and transcorneal penetration ($k_{in}$), as well as for activity against carbonic anhydrase ($I_{50}$). A number of these compounds exhibited lower sulfonamide p$K_a$ and higher water solubility than those of acetazolamide (1) and methazolamide (2), and one, 12, brought about a small reduction in IOP in the normal rabbit eye.

An important treatment of the abnormally high intraocular pressure (IOP) associated with glaucoma is by the suppression of aqueous humor formation via inhibition of ciliary process carbonic anhydrase (CA). 1,3,4-Thiadiazole-2-sulfonamides are among the most potent CA inhibitors with enzyme–inhibitor binding constants ($I_{50}$) in the $10^{-8}$–$10^{-9}$ M range. However, sulfonamides in current clinical use by the oral route, such as acetazolamide (5-(acetylamino)-1,3,4-thiadiazole-2-sulfonamide, 1) and methazolamide (5-(acetylimino)-4-methyl-$\Delta^2$-1,3,4-thiadiazoline-2-sulfonamide, 2), do not penetrate the cornea very well.[1] It has been shown that some 10–20 $\mu$M of free sulfonamide (of $I_{50}$ = $10^{-8}$ M) at the ciliary process is necessary for complete inhibition of the enzyme and lowering of pressure.[2] However, parenteral administration of these agents causes side effects such as paresthesias, numbness and tingling, fatigue, and depression,[3] so their usefulness as ocular hypotensive agents is somewhat limited.



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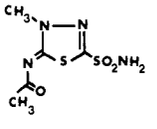
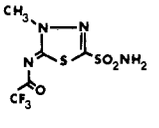


2



3

The suppression of aqueous humor formation by topical administration of carbonic anhydrase inhibitors was previously demonstrated by Maren et al.[1] for several 5-haloalkyl derivatives of 1 and 2. One of these, 5-[(trifluoroacetyl)imino]-4-methyl-$\Delta^2$-1,3,4-thiadiazoline-2-sulfonamide (3), possessed the requisite physicochemical properties to be topically effective in lowering IOP. A single drop of a 2% suspension lowered intraocular pressure in rabbit eye by 3.1 mmHg.[4] Spontaneous hydrolysis of the trifluoroacetyl group, however, precluded further development of this compound. Higher 5-alkyl homologues of 2 have recently been shown by Maren et al.[4] to produce similar reductions in IOP in the rabbit eye for several hours after administration of 1 drop (2% suspension).

Recently, a number of bicyclic sulfonamides related to benzothiazole-2-sulfonamide have shown promise as topically effective hypotensive agents.[5] In addition, Sugrue et al.[6] and Bar-Ilan et al.[7] have demonstrated IOP lowering in the rabbit after topical administration of 2-sulfamoyl-6-benzothiazolyl 2,2-dimethylpropionate, a prodrug that is enzymatically cleaved to 6-hydroxybenzothiazole-2-sulfonamide inside the eye.

**Chemistry.** In the present research we report the synthesis and physicochemical properties of a series of sulfonamides derived from 1,3,4-thiadiazolo[3,2-*a*]pyrimidine, 1,3,4-thiadiazolo[3,2-*a*]triazine, and related ring systems. All were prepared from the readily obtainable 5-amino-1,3,4-thiadiazole-2-sulfonamide (4) by annulation to the 1,3,4-thiadiazole nucleus at the 4,5-positions. The inclusion of ring heteroatoms such as N and S, as well as electron-withdrawing functionalities, i.e., carbonyl and sulfone, was anticipated to yield intermediates such as 6 and 18, which would show high intrinsic water solubility. These thiadiazolo[3,2-*a*]triazinesulfonamides could then be modified via the 5-position of the triazine ring to give less water soluble, more lipophilic derivatives with low sulfonamide p$K_a$. Simple thiadiazolo[3,2-*a*]triazinesulfonamides could also be obtained from the corre-

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