(9) R. W. Alexander, J. N. Davis, and R. J. Lefkowitz, *Nature (London)*, **258**, 437 (1975).
(10) L. T. Williams, R. Snyderman, and R. J. Lefkowitz, *J. Clin. Invest.*, **57**, 149 (1976).
(11) L. T. Williams, L. Jarett, and R. J. Lefkowitz, *J. Biol. Chem.*, **251**, 3096 (1976).
(12) C. Mukherjee, M. G. Caron, D. Mullikin, and R. J. Lefkowitz, *Mol. Pharmacol.*, **12**, 16 (1976).
(13) L. Limbird, P. De Meyts, and R. J. Lefkowitz, *Biochem. Biophys. Res. Commun.*, **64**, 1160 (1975).
(14) G. D. Aurbach, S. A. Fedak, C. J. Woodard, J. S. Palmer, D. Hauser, and F. Troxler, *Science*, **186**, 1223 (1974).
(15) E. M. Brown, D. Hauser, F. Troxler, and G. D. Aurbach, *J. Biol. Chem.*, **251**, 1232 (1976).
(16) M. E. Maguire, R. A. Wiklund, H. J. Anderson, and A. G. Gilman, *J. Biol. Chem.*, **251**, 1221 (1976).
(17) K. Biemann, "Mass Spectrometry", McGraw-Hill, New York, N.Y., 1962, p 241.
(18) J. M. A. Al-Rawi, J. P. Bloxsidge, J. A. Elvidge, J. R. Jones, V. E. M. Chambers, V. M. A. Chambers, and E. A. Evans, *Steroids*, **28**, 359 (1976).
(19) L. J. Altman and N. Silberman, *Anal. Biochem.*, in press.
(20) Y. Cheng and W. H. Prusoff, *Biochem. Pharmacol.*, **22**, 3099 (1973).
(21) R. F. Furchgott, *Ann. N.Y. Acad. Sci.*, **139**, 553 (1967).
(22) R. J. Lefkowitz, *J. Biol. Chem.*, **249**, 6119 (1974).
(23) R. J. Lefkowitz and M. G. Caron, *J. Biol. Chem.*, **250**, 4418 (1975).
(24) Y. Salomon, C. Londos, and M. Rodbell, *Anal. Biochem.*, **58**, 541 (1974).
(25) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

Pyrrole Esters of Tropanols and Related Structures as Analgesics[1]

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2,4,5-Trimethylpyrrole-3-carboxylic acid esters of tropanols and related monocyclic amino alcohols were synthesized and evaluated for analgesic activity by the mouse hot-plate and Nilsen methods. 1-Methyl-4-piperidinol 4-(2,4,5-trimethylpyrrole-3-carboxylate) (**7**) exhibited activity in the morphine–codeine range (mouse hot plate). In monkeys, **7** acted neither as a typical narcotic agonist nor as a typical antagonist and it showed no physical dependence liability of the morphine-type. Whereas the pethidine and prodine analgesics have quaternary phenyl substitution at C-4 of the piperidine ring, compound **7** does not.

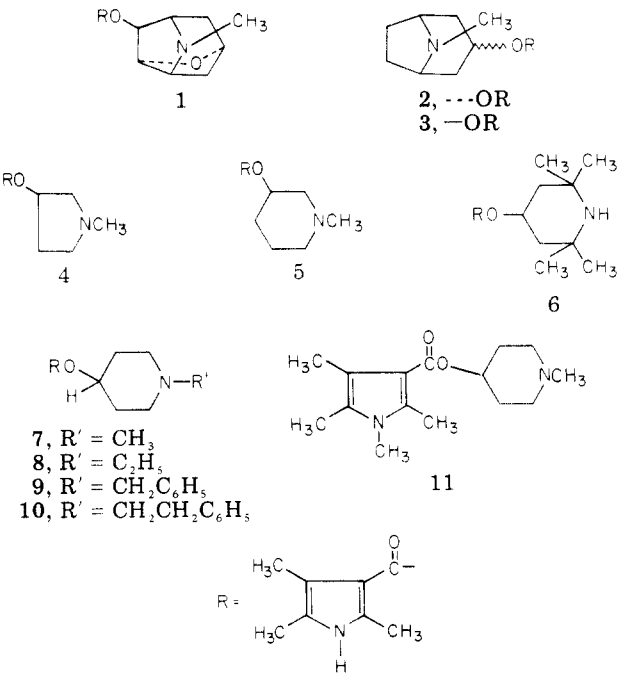
In an effort to develop new analgesics that are devoid of undesirable side effects and to study their interactions with stereospecific receptors, it was of interest to prepare pyrrolecarboxylates of tropanols and monocyclic amino alcohols related to scopoline ester **1** [3α,6α-epoxy-7β-hydroxytropane 7-(2,4,5-trimethylpyrrole-3-carboxylate)].[2] The scopoline ester **1** was observed to have analgesic activity comparable to that of codeine by the mouse hot-plate method, whereas the unesterified scopoline was inactive. In this investigation, epimeric tropanol esters **2** and **3**, pyrrolidinol ester **4**, and piperidinol esters **5**–**11** (Chart I and Table I) were prepared by the mixed trifluoroacetic anhydride procedure,[2] the preferred method for the synthesis of these labile pyrrole compounds.

Pharmacological Results and Discussion. The 2,4,5-trimethyl- and 1,2,4,5-tetramethylpyrrole-3-carboxylates were assayed for analgesic activity by the mouse hot-plate[3] and Nilsen methods[4] (Table II). The 3β-tropanol ester **3** exhibited an ED_{50} of 21.3, whereas the 3α-epimer **2** was only marginally active at 50 mg/kg. The 3-pyrrolidinol ester **4**, a partial structure of scopoline ester **1**, was marginally active (ED_{50} 64.3), while the 3-piperidinol ester **5** was inactive. The 1-methyl-4-piperidinol pyrrolecarboxylate **7** showed an ED_{50} of 4.9 (hot plate) and an ED_{50} of 7.1 (Nilsen). It was the most active compound in this study, with potency in the codeine–morphine range (hot-plate assay). The *N*-benzyl **9** and *N*-phenethyl **10** analogues were somewhat less active than *N*-methyl **7**. The tetramethylpyrrole compound **11**, analogous to **7**, was marginally active.

4-Piperidinol ester **7** showed no morphine-like dependence liability in monkeys.[5] In single dose suppression experiments, no suppression of the narcotic abstinence syndrome was indicated at 5.0 mg/kg. Compound **1** exhibited very mild CNS depression but it neither precipitated nor suppressed the withdrawl syndrome (3.0–24.0 mg/kg). In nonwithdrawn monkeys, **7** did not precipitate abstinence signs at 5.0 mg/kg.

A structure–activity comparison of the monocyclic amino alcohol esters **4**, **5**, and **7** indicates that the distance(s) between the heterocyclic pyrrole ring or the carbonyl bond (or conceivably both) and the protonated nitrogen of the piperidine ring in the more potent ester **7** is preferred for optimum receptor binding over those distances found in

Chart I[a]



[a] These compounds were obtained as salts as indicated in Table I.

Table I. Pyrrolecarboxylates of Tropanols and Related Structures

Compd	Mp, °C	Formula	Recrystn solvent[a]	Yield, %	Analyses
2	202-203	$C_{16}H_{24}N_2O_2 \cdot F_3CCOOH$	A	43.8	C, H, N
3	158-160	$C_{16}H_{24}N_2O_2 \cdot F_3CCOOH \cdot 0.5H_2O$	B	19.6	C, H, N
4	103-108[b]	$C_{13}H_{20}N_2O_2 \cdot HCl \cdot 0.25H_2O$	D	60.7	C, H, N
5	152-158[b]	$C_{14}H_{22}N_2O_2 \cdot HCl \cdot 0.5H_2O$	D	28.2	C, H, N
6	254-256	$C_{17}H_{28}N_2O_2 \cdot F_3CCOOH$	C	92.1	C, H, N
7	182-183	$C_{14}H_{22}N_2O_2 \cdot F_3CCOOH$	B	35.5	C, H, N
8	166-167	$C_{15}H_{24}N_2O_2 \cdot F_3CCOOH$	A	28.7	H, N; C[c]
9	157-170[b]	$C_{20}H_{26}N_2O_2 \cdot HCl \cdot H_2O$	D	41.3	C, H, N
10	182-184	$C_{21}H_{28}N_2O_2 \cdot F_3CCOOH$	A	33.8	C, H, N
11	180-184	$C_{15}H_{24}N_2O_2 \cdot F_3CCOOH$	A	41.7	C, H, N

[a] A = acetone, B = acetone-ether, C = acetone-MeOH, D = acetone-ether precipitation. [b] Obtained as an amorphous, hygroscopic solid; the melting point was not sharp. [c] C: calcd, 53.96; found, 53.44.

Table II. Analgesic Activity

Compd[a]	ED_{50}, mg/kg[b] Hot plate	Nilsen
1	6.0 (4.6-7.7)	28.9 (21.8-38.3)
2	Marginally act. at 50	
3	21.3 (14.0-32.3)	
4	64.3 (39.6-104.4)	
5	Inact. at 100	
6	Inact. at 100	
7	4.9 (3.6-6.5)	7.1 (5.3-9.4)
8	10.4 (7.3-15.0)	
9	6.3 (4.3-9.2)	
10	Marginally act. at 20	
11	Marginally act. at 20	
Codeine	7.5 (6.7-8.3)	4.5 (2.7-7.6)
Morphine	1.2 (0.9-1.3)	0.8 (0.6-1.2)

[a] Tested subcutaneously as water-soluble HCl or trifluoroacetic acid salts as indicated in Table I. [b] Numbers in parentheses are the 95% confidence limits obtained by probit analysis.

compounds 4 and 5 (marginally active and inactive, respectively). Since compounds 6 and 11, essentially inactive, have the 4-piperidinol nucleus present in 7, while the scopoline ester 1, nearly equipotent as 7, is geometrically different, such factors as lipophilicity (ability to cross the blood–brain barrier) and metabolic disposition may also play important roles in governing the activity of this series. Pethidine and alphaprodine are well-known piperidine type analgesics that have morphine-like side effects, including physical dependence liabilities.[6,7] It should be noted that 7 differs from pethidine and alphaprodine in that it does not have quaternary C-4 phenyl substitution of the piperidine ring.

Further work on other heterocyclic and aromatic carboxylates of this nonquaternary 4-piperidinol ring system, including substituent effects and spatial requirements for receptor binding, is in progress. Also, since large potency factors have been noted in the diastereomeric pairs of the prodine,[8-10] promedol,[11] and pethidine[12] series, isomers of this piperidinol ring will be investigated.

Experimental Section

Melting points were taken on a Kofler hot stage and are corrected. Analytical results obtained were within ±0.4% of the theoretical values. Infrared spectra were obtained on a Perkin-Elmer spectrometer Model 237B.

Pyrrolecarboxylates. The mixed trifluoroacetic anhydride method[2] was used for the preparation of these esters. The general procedure used will be described. To a magnetically stirred mixture of 6 mmol of 2,4,5-trimethylpyrrole-3-carboxylic acid[2,13] or 1,2,4,5-tetramethylpyrrole-3-carboxylic acid in 18 mL of dry benzene was added 6 mmol of trifluoroacetic anhydride at room temperature. After a 5-min reaction period, 6 mmol of the tropanol, piperidinol, or pyrrolidinol in 6 mL of dry benzene was added to the mixed anhydride solution. The reaction mixture was then stirred for 1–2 h at room temperature, and the solvent was removed in vacuo, followed by two additional evaporations from benzene. The crude reaction mixture was chromatographed on silica gel (elution with increasing percentages of MeOH in CH_2Cl_2). Homogeneous fractions, as indicated by TLC ($CHCl_3$–MeOH, 9:1; silica gel GF) and appropriate ester bands in the infrared, were combined and evaporated and the products recrystallized from the designated solvent (Table I) to yield the basic ester as the trifluoroacetic acid salt. Compounds 4, 5, and 9 were converted to the free bases and obtained as the HCl salts with ethereal HCl.

Ethyl 1,2,4,5-Tetramethylpyrrole-3-carboxylate. To 1.05 g of small, freshly cut pieces of sodium in 60 mL of dry toluene was added 5.0 g (0.028 mol) of ethyl 2,4,5-trimethylpyrrole-3-carboxylate. The magnetically stirred mixture was heated on an oil bath (105 °C) for 4 h. A brown solid precipitated after ca. 45 min of heating. To the mixture was then added 3.4 mL of dimethyl sulfate dropwise over a period of 15 min and heating was continued for 90 min. The reaction mixture was cooled and the brown precipitate removed by suction filtration. After washing the precipitate with toluene, the filtrate was evaporated in vacuo. The residue was mixed with ethanol and evaporated and the product recrystallized from dilute MeOH to give 2.365 g (43.3%) of ethyl 1,2,4,5-tetramethylpyrrole-3-carboxylate as a tan crystalline solid, mp 71–72.5 °C. Anal. ($C_{11}H_{17}NO_2$) C, H, N.

1,2,4,5-Tetramethylpyrrole-3-carboxylic Acid. A solution of 717 mg (3.67 mmol) of ethyl 1,2,4,5-tetramethylpyrrole-3-carboxylate in 18 mL of 20% ethanolic KOH was refluxed for 18 h. The solvent was removed in vacuo and the residue dissolved in 22 mL of H_2O. After filtration to remove some insoluble material, the filtrate was cooled in ice and then acidified with 55 mL of 5% HCl (to congo red). The precipitate was collected by suction filtration, washed with water, and air-dried to give 580 mg (94.5%) of the tetramethylcarboxylic acid as a tan solid. Recrystallization from ethanol gave an analytical sample, mp 229–231 °C dec. Anal. ($C_9H_{13}NO_2$) C, H, N.

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References and Notes

(1) Presented in part at the 172nd National Meeting of the American Chemical Society, San Francisco, Calif., August 30, 1976, MEDI 24.
(2) J. A. Waters, C. R. Creveling, and B. Witkop, *J. Med. Chem.*, 17, 488 (1974).
(3) A. E. Jacobson and E. L. May, *J. Med. Chem.*, 8, 563 (1965).
(4) T. D. Perrine, L. Atwell, I. B. Tice, A. E. Jacboson, and E. L. May, *J. Pharm. Sci.*, 61, 86 (1972).
(5) Committee on Problems of Drug Dependence, *Bull. Narc.*, 25 (no. 2), 25 (1973).
(6) A. F. Casy, *Prog. Med. Chem.*, 7, 229 (1970).
(7) A. E. Jacobson, E. L. May, and L. J. Sargent in "Medicinal Chemistry", 3rd ed, Part II, A. Burger, Ed., Wiley, New York, N.Y., 1970, p 1327.

(8) P. S. Portoghese and E. Shefter, J. Med. Chem., 19, 55 (1976).
(9) K. H. Bell and P. S. Portoghese, J. Med. Chem., 16, 203 (1973).
(10) M. A. Iorio, A. F. Casy, and E. L. May, Eur. J. Med. Chem., 10, 178 (1975).
(11) D. S. Fries and P. S. Portoghese, J. Med. Chem., 19, 1155 (1976).
(12) A. H. Beckett, A. F. Casy, and G. Kirk, J. Med. Pharm. Chem., 1, 37 (1959).
(13) L. Knorr and K. Hess, Justus Liebigs Ann. Chem., 236, 317 (1886).

Potential Steroidal Antiestrogens

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A series of analogues of 17β-estradiol has been synthesized and the compounds have been tested, using sucrose density gradient analysis, for their ability to compete with [6,7-^{3}H]-17β-estradiol for the estrogen-receptor protein from mouse uterine homogenates. Active compounds were also tested for antiuterotrophic activity in immature rats and/or mice. 3,17β-Dihydroxy-6-phenylestra-1,3,5(10),6-tetraene (**14**) was the most active new compound in the in vitro test suppressing the binding of 17β-estradiol by 34 and 87%, respectively, at molar ratios of 1 and 3.16. It was significantly more potent than the intermediate 6-oxoestradiol (**4**) which produced a 52% inhibition of binding at a molar ratio of 3.16. The thiosemicarbazone of 6-oxoestradiol (**17**) and the derived 3,17β-dihydroxy-6-(2-imino-4-oxothiazolidinyl-1-imino)estra-1,3,5(10)-triene (**19**) produced, respectively, only 46 and 16% inhibition of binding at a molar ratio of 10. Introduction of a 1-methyl substituent into either 6-oxo or 6-phenyl compounds reduced affinity for the receptor significantly (compounds **5** and **15**) and conversion of the 3-OH into a β-dialkylaminoethoxy group virtually destroyed all binding activity (compounds **2**, **6**, **10**, and **11**). At a molar ratio of 10 compound **14** failed to suppress the uterine weight response of immature rats to 17β-estradiol, whereas compound **15**, at a molar ratio of 200, produced a significant increase in the uterine weight of immature rats but not of immature mice even at a molar ratio of 1000.

Hoover et al.[1] concluded from a survey of 45 853 cases of breast cancer that the risk of endometrial cancer is increased by treatment with hormones. During the period covered by this survey (1935–1971) the hormones used are believed to have been mainly nonsteroidal, e.g., diethylstilbestrol, which has also been linked with carcinoma of the genital tract in daughters of mothers treated with the drug during pregnancy[2,3] and in young women receiving the drug for ovarian agenesis.[4] Currently available antiestrogens are related to the nonsteroidal triphenylethylene group of estrogens and possess weak estrogenic activity,[5] a property which would appear to be highly undesirable if the drug is to be administered over a long period in the treatment of hormone-dependent carcinoma of the breast.

Among other classes of drugs, e.g., cholinomimetics, histamine, and sympathomimetics, the introduction of large nonpolar substituents into an agonist often leads to a loss of agonistic and the development of antagonistic properties.[6] In a search for new antiestrogens which lack estrogenic activity we have synthesized a number of novel ring A aromatic steroids in which the nucleus is substituted with phenyl, benzyl, and 2-imino-4-oxothiazolidinyl-1-imino groups. The effects of replacing the 3-hydroxyl by β-dialkylaminoethoxy and the introduction of the 1-methyl substituent in these modified steroids have also been studied.

The affinities of the new compounds for the estrogen receptor protein have been determined in vitro by measuring their ability to compete with tritium labeled 17β-estradiol, and compounds with significant binding activity have been tested in vivo for antiuterotrophic activity in rats and/or mice.

Chemistry. The required compounds were synthesized via the routes shown in Scheme I. Chromic acid oxidation of 17β-estradiol diacetate (**3**) followed by hydrolysis yielded the key intermediate 6-oxoestradiol[7] (**4**). The position of the carbonyl group was confirmed by the aryl ketone band (ν 1680 cm^{-1}) in the IR spectrum, the downfield shift of the C_4-H signal in the NMR spectrum to δ 7.41 due to the deshielding of the adjacent carbonyl group, and the similarity of the UV spectrum to that of the 1-methyl homologue **5**, which was synthesized from 3,17β-dihydroxy-1-methylestra-1,3,5(10),6-tetraene[8] (**1**) by the method of Pelc.[9]

β-Diethylaminoethyl chloride or its dimethyl analogue with compound **1** or ketones **4** or **5** in the presence of sodium methoxide yielded the dialkylaminoethyl ethers **2** and **6–9** as oils which were purified as their hydrochlorides. The salts were very hygroscopic and retained water of crystallization even after drying at raised temperature and reduced pressure (Table I).

Reactions of these ketonic ethers **6–9** with phenylmagnesium bromide or benzylmagnesium chloride gave the required aminoalkyl ethers **10–13** and the related free phenols **14** or **15** were obtained by reacting phenylmagnesium bromide with phenolic ketones **4** and **5**, respectively. The double bond in the 6-benzyl-substituted compound **13** was assigned endocyclic from a comparison of its UV spectrum with that of the 6-phenyl compounds and by the presence of signals due to the methylene protons of benzyl and a single C_7-proton in the NMR spectrum. 6-Oxoestradiol diacetate (**4** diacetate) was converted into its thiosemicarbazone **16** which condensed with ethyl bromoacetate to form 3,17β-acetoxy-6-(2-imino-4-oxothiazolidinyl-1-imino)estra-1,3,5(10)-triene (**18**). The free phenolic thiosemicarbazone **17** with ethyl bromoacetate gave the corresponding dihydroxy derivative **19**. The NMR spectrum of the thiazolidinyl compound **18** was consistent with the assigned structure, the C_4-H signal being shifted downfield to δ 7.71 due to the deshielding of the exocyclic double bond at C_6. The UV spectra of diol **19** and diacetate **18** were very similar and consistent with the assignment of the analogous 6-thiazolidinylimino structure to the diol **19**.

Biological Results. The ability of the compounds to compete with [6,7-^{3}H]-17β-estradiol for the cytoplasmic estrogen-receptor protein isolated from mouse uterine