(log ϵ 4.16) nm; *m/e*: 311 (M^+).

Anal.—Calc. for $C_{19}H_{21}NO_3$: C, 73.31; H, 6.75; N, 4.50. Found: C, 73.21; H, 6.81; N, 4.58.

4. Alpinigenine: R_f 0.68, mp 185–186° [lit. (2) mp 185–186°], mixed melting point with an authentic sample 185–186°; $[\alpha]_D^{20}$ +230.2° (c = 1.59, $CHCl_3$) [lit. (15) $[\alpha]_D^{20}$ +286°]. The spectral data (IR, UV, NMR, and mass) were identical with an authentic sample (2).

The alkaloid profiles of *P. orientale* L. from different geographical areas suggested classification according to five different chemotypes (Table I). Oripavine was either a sole alkaloid of the poppy (chemotype A) or a single major alkaloid (chemotypes B, C, D, and E).

A literature survey (16) revealed that alpinigenine was not found previously in *P. orientale* L.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 22, 1976, from the *Institute of Medicinal Plants, College of Pharmacy, Tehran University, Tehran, Iran.*

Accepted for publication August 25, 1976.

Supported by the Iranian Ministry of Science and Higher Education Research Development Center.

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Physiologically Active Substances from Marine Sponges IV: Heterocyclic Compounds

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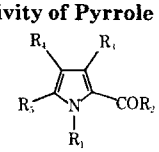
Abstract □ Several guanidine compounds were synthesized by the reaction of acid chlorides of thiophene and furan with guanidines. Some of these compounds showed antibiotic and cytotoxic activities. Series of pyrrole compounds were synthesized and found to have significant antibiotic activity.

Keyphrases □ Pyrroles, various—synthesized, evaluated for antibacterial activity □ Furans, various—synthesized, evaluated for antibacterial and cytotoxic activity □ Thiophenes, various—synthesized, evaluated for antibacterial and cytotoxic activity □ Heterocycles—various pyrroles, furans, and thiophenes synthesized, evaluated for antibacterial and cytotoxic activity □ Antibacterial activity—various pyrroles, furans, and thiophenes evaluated □ Cytotoxic activity—various furans and thiophenes evaluated □ Structure–activity relationships—various pyrroles, furans, and thiophenes evaluated for antibacterial and cytotoxic activity

Several biologically significant pyrrole (1) and indole (2, 3) derivatives have been isolated from marine sources. Most of these compounds show pronounced antibiotic activity. During the present investigation of the sponge *Agelas* sp. (JC-6), a new brominated pyrrole derivative incorporating the guanidine moiety was isolated and showed significant activity against *Escherichia coli* (4). This natural product also showed cytotoxicity against KB carcinoma cells (5). This report describes the synthesis of several pyrrole, furan, and thiophene analogs and their physiological activities.

Table I—Antibiotic Activity of Pyrrole Derivatives



Compound	R_1	R_2	R_3	R_4	R_5	Activity against *E. coli*[a]
XII*a*	H	OCH_3	H	H	H	+++
XII*b*	H	NH_2	H	H	H	++++
XII*c*	CH_3	OCH_3	H	H	H	+++
XII*d*	CH_3	NH_2	H	H	H	++++
XII*e*	H	H	H	Br	H	++++
XII*f*	H	OCH_3	H	Br	H	++++
XII*g*	H	OCH_3	H	Br	Br	++
XII*h*	H	OCH_3	Br	Br	Br	+

[a] After paper disk incubation, zones of bacterial inhibition were measured: +, <15 mm; ++, 15–18 mm; +++, 18–22 mm; and ++++, >22 mm.

EXPERIMENTAL[1]

Chemistry—Since 2-aminopyrimidine (I*a*) and guanidine (II*a*) have a common structural unit, it was considered desirable to synthesize furan, thiophene, and pyrrole derivatives incorporating this structural feature.

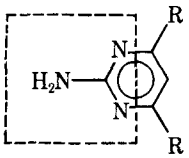
[1] All melting points are uncorrected. Microanalyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N.Y. IR spectra were recorded on a Perkin-Elmer 421 grating IR spectrometer, NMR spectra were recorded on a Varian A-60A spectrometer, and mass spectra were recorded on a Hitachi Perkin-Elmer spectrometer 107.

Table II—Analytical and Spectral Data

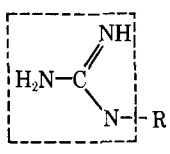
Compound	Melting Point	Yield, %	Formula		Analysis, % Calc.	Found	Spectral Data
V	290–293°	18	$C_8H_{11}N_3O_2 \cdot H_2O$	C	48.23	47.87	IR (KBr): 1680 (CO) cm^{-1}; NMR (CD_3OD): δ 3.03 (s, 6H), 6.43 (m, 1H), 6.85 (m, 1H), and 7.47 (m, 1H)
				H	6.58	6.45	
				N	21.09	20.82	
VII	124–127°	25	$C_9H_7N_3O_2$	C	57.14	57.20	IR (mineral oil): 3420 (NH) and 1680 (CO) cm^{-1}; NMR ($CDCl_3$): δ 7.1 (m, 3H), 7.6 (m, 3H), and 8.7 (m, 2H); mass spectrum (M^+): *m/e* 189
				H	3.73	3.96	
				N	22.21	22.23	
VIII	183–185°	20	$C_{11}H_{11}N_3O_2$	C	60.82	60.77	IR (KBr): 3450 (NH) and 1680 (CO) cm^{-1}; NMR ($CDCl_3$): δ 2.45 (s, 6H), 6.55 (m, 1H), 6.78 (s, 1H), 7.3 (m, 1H), and 7.51 (m, 1H); mass spectrum (M^+): *m/e* 217
				H	5.10	5.09	
				N	19.34	19.05	
VI	269–270°	19	$C_8H_{11}N_3O_2S \cdot 2H_2O$	C	41.20	41.67	IR (KBr): 3340 and 3290 (NH, H_2O) cm^{-1}; mass spectrum (M^+): *m/e* 215
				H	6.24	5.94	
				N	18.02	18.30	
				S	13.44	13.72	
IX	160–161°	35	$C_9H_7N_3OS$	C	52.69	52.99	IR (KBr): 3450 (NH) and 1670 (CO) cm^{-1}; NMR ($CDCl_3$): δ 7.1 (m, 3H), 7.65 (m, 3H), and 8.96 (m, 2H); mass spectrum (M^+): *m/e* 205
				H	3.44	3.35	
				N	20.48	20.73	
				S	15.59	15.71	
X	168–169°	37	$C_{11}H_{11}N_3OS$	C	56.65	56.49	IR (KBr): 3450 (NH) and 1670 (CO) cm^{-1}; NMR ($CDCl_3$): δ 7.1 (m, 3H) and 7.5 (m, 2H); mass spectrum (M^+): *m/e* 233
				H	4.75	4.80	
				N	18.02	18.10	
				S	13.72	13.89	
XI	153–154°	5	$C_{14}H_9N_3O_2S$	C	53.34	53.59	IR (KBr): 3450 (NH) and 1670 (CO) cm^{-1}; NMR ($CDCl_3$): δ 8.75 (m, 2H), 7.6 (m, 4H), and 7.05 (m, 3H); mass spectrum (M^+): *m/e* 283
				H	2.88	2.79	
				N	13.34	13.60	
				S	20.20	19.90	

The reaction of guanidine (II*a*) with 2-furoyl chloride (III) and 2-thiophenecarbonyl chloride (IV) afforded intractable mixtures. However, the reaction of *N,N*-dimethylguanidine (II*b*) with these two carbonyl chlorides yielded crystalline derivatives V and VI.

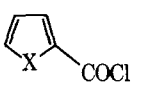
2-Aminopyrimidine (I*a*) and 2-amino 4,6-dimethylpyrimidine (I*b*), on similar treatment with these acid chlorides, resulted in products VII–X. The 5-acylated derivative (XI) was also isolated as a minor component from the reaction of 2-aminopyrimidine (I*a*) and thiophene carbonyl chloride (IV). This product resulted from the nucleophilic substitution at C-5 of the pyrimidine ring. Attempts to synthesize similar derivatives from pyrrole were unsuccessful.



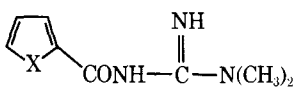
I*a*: R = H
I*b*: R = CH_3



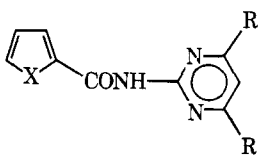
II*a*: R = H_2
II*b*: R = $(CH_3)_2$



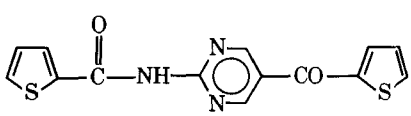
III: X = O
IV: X = S



V: X = O
VI: X = S



VII: X = O, R = H
VIII: X = O, R = CH_3
IX: X = S, R = H
X: X = S, R = CH_3



XI

Biological Activity—The thiophene and furan derivatives were tested for antibacterial (4) and cytotoxic activities (6).

In testing the activity against *Escherichia coli*[2] (ATCC 4157), 0.005 g of the compound was applied to a paper disk and incubated for 18 hr at 35°. Zones of bacterial inhibition were measured. Compounds IX and X showed 15–18-mm inhibition zones; VI showed an 18–22-mm zone.

Cytotoxic activity was tested against human oral epidermoid carcinoma KB cells (ATCC CCL 17) by adding 25–50 μg/ml of the test compound to the growth medium. Compounds V and VI showed 50% cytotoxicity.

Furan derivatives V, VII, and VIII were inactive against *E. coli*, but V was fairly cytotoxic. Thiophene derivatives VI, IX, and X showed moderate activity against *E. coli.*, but only VI exhibited cytotoxic activity.

Several pyrrole derivatives were also examined for antibacterial activity (Table I) and were considerably more potent as antibacterial agents than the corresponding thiophene and furan compounds. Although it is difficult to establish rigorously any structure–activity relationship, the introduction of halogen progressively decreased the antibiotic activity in this series.

General Method for Synthesis of Furan and Thiophene Derivatives—A solution of 2-furoyl chloride (III) (3.92 g, 0.03 mole) was added dropwise, at room temperature over 2 hr, to a suspension of 2-amino-4,6-dimethylpyrimidine (I*b*) (3.69 g, 0.03 mole) and triethylamine (3.03 g, 0.03 mole) in 150 ml of acetonitrile. The reaction mixture was heated to 65° for 0.5 hr and then kept overnight at room temperature. The precipitated triethylamine hydrochloride was filtered off, and the filtrate was evaporated under reduced pressure.

The residue was dissolved in chloroform (150 ml), washed with sodium bicarbonate solution (50 ml, 1 *N*) and water (2 × 100 ml), and dried over magnesium sulfate. Removal of the solvent afforded a dark viscous material. Repeated crystallization from ethyl acetate gave VIII.

Analytical and spectral data for these compounds are given in Table II.

Pyrrole derivatives were synthesized by literature methods (7–9).

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 10, 1976, from the *Osborn Laboratories of Marine Sciences, New York Aquarium, New York Zoological Society, Brooklyn, NY 11224.*

Accepted for publication August 27, 1976.

Supported by Sea Grant Project Grant 1-35263 to the New York Zoological Society.

The authors thank Professor A. K. Bose of Stevens Institute of Technology, Hoboken, N.J., for helpful discussions and for providing facilities for the recording of mass and NMR spectra.

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Preparation of Spin-Labeled Opiates: Morphine and Codeine

JAMES A. CELLA [x] and JAMES A. KELLEY

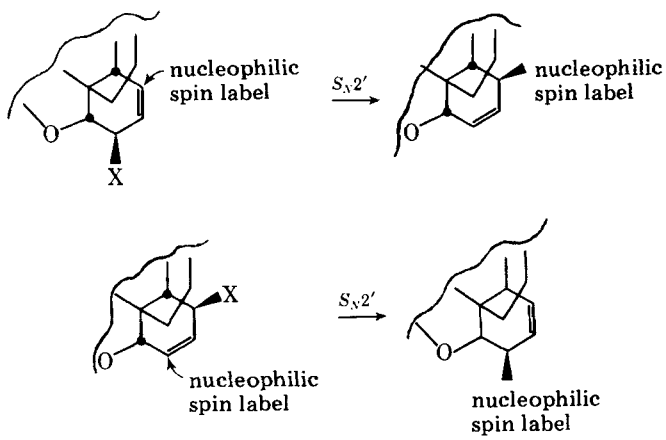
Abstract □ The preparation of 6-spin-labeled codeine and morphine is described. Treatment of either 6-chlorocodide or 8-bromocodide with 4-amino-2,2,6,6-tetramethylpiperidino-1-oxyl free radical in dimethylformamide afforded 6-spin-labeled codeine. Similar treatment of 6-chloromorphide afforded 6-spin-labeled morphine. Exclusive formation of the 6-isomer in these reactions is explained by halide-ion-catalyzed isomerization of the 6-halo opiate to the 8-halo isomer followed by a normal S_N2' displacement of the halogen. Both spin-labeled compounds displayed weak *in vivo* analgesic activity and did not bind appreciably to receptors in brain homogenate.

Keyphrases □ Spin-labeled opiates—morphine and codeine labeled in 6-position, effect on binding to brain receptors and analgesic activity □ Morphine—spin labeled in 6-position, effect on binding to brain receptors and analgesic activity □ Codeine—spin labeled in 6-position, effect on binding to brain receptors and analgesic activity □ Opiates—morphine and codeine, spin labeled in 6-position, effect on binding to brain receptors and analgesic activity □ Analgesics, narcotic—morphine and codeine, spin labeled in 6-position, effect on binding to brain receptors and analgesic activity

In numerous attempts to identify and characterize the opiate receptor (1–4), the binding characteristics of pharmacologically active and inactive isomers were compared or the reduced agonist binding in the presence of antagonists was noted using radiolabeled opiates with high specific activity. The spin label method (5, 6) is an attractive alternative to the radioisotope method for studying this drug–receptor interaction *in vitro*, since it obviates the need for separating bound from unbound drug. A preliminary report demonstrated the feasibility of this method (7).

DISCUSSION

Essential to the success of this approach is the requirement that the nitroxide label not constitute a significant perturbation to the interaction of the drug with its receptor. As such, the label should be attached to a pharmacologically unimportant position in the drug molecule. For purposes of stability, the label should be covalently attached to the drug *via* a linkage resistant to hydrolytic enzymes and inert to aqueous media at physiological pH. Finally, it is desirable, although not essential, that the labeled drug possess biological activity similar to that of the parent drug.



Scheme I

With these requirements in mind, the preparation of spin-labeled derivatives of morphine and codeine suitable for the *in vitro* study of the interaction of these drugs with possible receptors was attempted.

The hydroxyl group at the 6-position of morphine and codeine appeared to offer a suitable point for attachment for the spin label, particularly in view of the relative unimportance of this position in the pharmacological activity of these drugs (8, 9). However, the steric inaccessibility of this alcohol (10, 11) precluded all attempts to alkylate at this position[1]. Consequently, the mode of reaction was reversed by using a nucleophilic spin label to displace halogen from a 6- or 8-halo opiate (Scheme I). In the isomeric halocodides and morphides, the halogen atom has a beta configuration (*syn* to the ethylamino bridge) and is displaced by nucleophiles exclusively from the beta side in an S_N2' fashion (13). Following this precedent, the reaction of the isomeric halocodides and morphides with 4-amino-2,2,6,6-tetramethylpiperidino-1-oxyl free radical (I) as a route to spin-labeled morphine and codeine was investigated.

Reaction of I with 8-bromocodide (IIa) in dimethylformamide afforded the expected 6-spin-labeled codeine (IIIa) in 23% yield (Scheme II).

[1] A recent report (12) described the selective methylation of morphine at the 6-position *via* its dipotassium salt. A similar approach to introduce a spin label at the 6-position was attempted; however, with alkylating agents much larger than methyl, reaction occurred exclusively at the 3-position.