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Synthesis and Antibacterial and Antifungal Activities of Alkyl and Polyhalophenyl Esters of Benzo[*b*]-3-methyl-2-furancarbamic Acid

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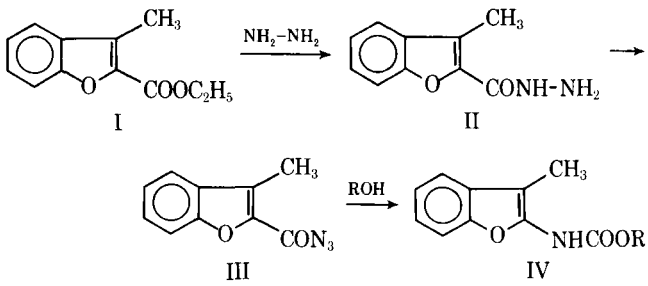
Abstract □ Several alkyl and polyhalophenyl esters of benzo[*b*]-3-methyl-2-furancarbamic acid were prepared and tested for antifungal activity against *Candida albicans, Penicillium notatum*, and *Aspergillus niger*. The pentachlorophenyl ester was the most active substance and the only compound to show antibacterial activity against *Staphylococcus aureus*.

Keyphrases □ Carbamic acid esters—synthesized, screened for antifungal and antibacterial activities □ Furancarbamic acid esters—synthesized, screened for antifungal and antibacterial activities □ Antifungal activity—alkyl and polyhalophenyl esters of furancarbamic acid synthesized and screened □ Antibacterial activity—alkyl and polyhalophenyl esters of furancarbamic acid synthesized and screened

In continuing studies on the chemistry and antibacterial and antifungal activities of carbamic acid esters (1–3), alkyl and polyhalophenyl esters of benzo[*b*]-3-methyl-2-furancarbamic acid were synthesized from benzo[*b*]-3-methyl-2-furancarboxazide (III) and the appropriate alcohol or phenol (Scheme I). The physical data of the compounds prepared are summarized in Table I. All compounds listed in Table I were tested against *Candida albicans* (28012), *Penicillium notatum* (S-13), and *Aspergillus niger* (23171) *in vitro* using Sabouraud dextrose agar medium[1].

Each compound was dissolved in acetone to a concentration of 1 mg/ml. These solutions were diluted with hot culture medium to the desired concentrations and autoclaved at 120° for 2 hr. Five replicates of each concentration were prepared.

The antifungal activity of all compounds tested, except IV*n*, was insignificant at a concentration of 5 μg/ml. All compounds were active against *P. notatum* and *A. niger* but inactive against *C. albicans* at a concentration of 10 μg/ml. However, they were active against *C. albicans* at a concentration of 30 μg/ml. Griseofulvin was used as a control (Table II).



Scheme I

All compounds also were tested against *Bacillus subtilis* (NCTC 3610), *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 10031), and *Sarcina lutea* (ATCC 9341). Nitrofurazone was used as a control. Standard paper disks, 6 mm in diameter, were immersed in solution and placed on an inoculated assay medium surface[2].

The antibacterial activity of all compounds that dissolved in acetone at the 0.5% concentration was insignificant. However, IV*n* at the same concentration showed 12-mm inhibition zones against *S. aureus* and had no activity against other strains.

EXPERIMENTAL[3]

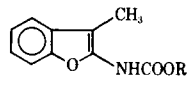
Benzo[*b*]-3-methyl-2-furancarboxyhydrazide (II)—To a stirring solution of 25 g (0.5 mole) of hydrazine hydrate in 150 ml of ethanol was added dropwise a solution of 20.4 g (0.1 mole) of ethyl benzo[*b*]-3-methyl-2-furancarboxylate (I) (5) in 50 ml of ethanol.

[1] These microorganisms were obtained from the Department of Parasitology, Public Health Institute, Tehran, Iran.

[2] Antibiotic assay medium, British Pharmacopoeia, 1968.

[3] Melting points were taken on a Kofler hot-stage microscope and are uncorrected. IR spectra were recorded using a Leitz model III spectrograph. NMR spectra were recorded on a Varian A60A instrument.

Table I—Physical Constants of Benzo[*b*]-3-methyl-2-furancarbamic Acid Esters



Compound	R	Yield, %	Melting Point[a]	Formula[b]	Analysis, %	
					Calc.	Found
IV*a*	CH_3	80	94°	$C_{11}H_{11}NO_3$	C 64.39 H 5.37 N 6.83	64.28 5.41 6.97
IV*b*	C_2H_5	75	80–81°[c]	—	—	—
IV*c*	n-C_3H_7	65	82–83°	$C_{13}H_{15}NO_3$	C 66.95 H 6.44 N 6.01	66.87 6.32 6.17
IV*d*	iso-C_3H_7	68	133–134°	$C_{13}H_{15}NO_3$	C 66.95 H 6.44 N 6.01	67.14 6.38 5.82
IV*e*	n-C_4H_9	50	78–79°	$C_{14}H_{17}NO_3$	C 68.02 H 6.88 N 5.67	68.14 6.72 5.52
IV*f*	C_6H_{11}	75	132–133°	$C_{16}H_{19}NO_3$	C 70.33 H 6.96 N 5.13	70.17 7.12 5.05
IV*g*	$CH_2C_6H_5$	70	104–105°	$C_{17}H_{15}NO_3$	C 72.60 H 5.34 N 4.98	72.75 5.51 4.99
IV*h*	*p*-Chlorophenyl	55	141–142°	$C_{16}H_{12}ClNO_3$	C 63.68 H 3.98 N 4.64	63.79 3.82 4.73
IV*i*	*p*-Bromophenyl	70	155–156°	$C_{16}H_{12}BrNO_3$	C 55.49 H 3.47 N 4.05	55.62 3.58 4.23
IV*j*	*o*-Chlorophenyl	55	130–131°	$C_{16}H_{12}ClNO_3$	C 63.68 H 3.98 N 4.64	63.53 3.99 4.75
IV*k*	2,4-Dichlorophenyl	45	155–156°	$C_{16}H_{11}Cl_2NO_3$	C 57.14 H 3.27 N 4.17	57.01 3.12 4.35
IV*l*	2,4,5-Trichlorophenyl	85	138–139°	$C_{16}H_{10}Cl_3NO_3$	C 51.82 H 2.70 N 3.78	52.03 2.85 3.85
IV*m*	2,4,6-Trichlorophenyl	83	162–163°	$C_{16}H_{10}Cl_3NO_3$	C 51.82 H 2.70 N 3.78	51.65 2.85 3.63
IV*n*	Pentachlorophenyl	90	169–170°	$C_{16}H_8Cl_5NO_3$	C 43.69 H 1.82 N 3.19	43.82 1.93 3.09

[a] Unless otherwise indicated, the recrystallization solvent was benzene or benzene–hexane. [b] IR and NMR spectra of all compounds were as expected. [c] Lit. (4) mp 81–82°.

After the addition was complete, the mixture was refluxed for 5 hr. The solvent was evaporated, and the residue was crystallized from ethanol to give 17 g (90%) of II, mp 139–140° [lit. (4) mp 139–141°].

Benzo[*b*]-3-methyl-2-furancarboxazide (III)—To a stirring solution of II (20.6 g, 0.1 mole) in 200 ml of 50% acetic acid at 0° was added dropwise a solution of sodium nitrite (6.9 g, 0.1 mole) in 100 ml of water. The reaction mixture was stirred for an additional 30 min. The precipitate was filtered, washed with water, and dried at room temperature under reduced pressure, mp 105–106° [lit. (4) mp 105–106.5°].

Methyl Benzo[*b*]-3-methyl-2-furancarbamate (IV*a*)—A solution of III (2.01 g, 0.01 mole) in 50 ml of absolute methanol was refluxed for 5 hr. The solvent was evaporated, and the residue was crystallized from benzene–hexane, mp 94–95°; IR (KBr): 3200 (NH),

Table II—Antifungal Activity[a] of Alkyl and Polyhalophenyl Esters of Benzo[*b*]-3-methyl-2-furancarbamic Acid

Compound	*P. notatum*			*C. albicans*			*A. niger*		
	5 μg/ml	10 μg/ml	30 μg/ml	5 μg/ml	10 μg/ml	30 μg/ml	5 μg/ml	10 μg/ml	30 μg/ml
IV*a*	—	+	+	—	—	+	—	+	+
IV*b*	—	+	+	—	—	+	—	+	+
IV*c*	—	+	+	—	—	+	—	+	+
IV*d*	—	+	+	—	—	+	—	+	+
IV*e*	—	+	+	—	—	+	—	+	+
IV*f*	—	+	+	—	—	+	—	+	+
IV*g*	—	+	+	—	—	+	—	+	+
IV*h*	—	+	+	—	—	+	—	+	+
IV*i*	—	+	+	—	—	+	—	+	+
IV*j*	—	+	+	—	—	+	—	+	+
IV*k*	—	+	+	—	—	+	—	+	+
IV*l*	—	+	+	—	—	+	—	+	+
IV*m*	—	+	+	—	—	+	—	+	+
IV*n*	+	+	+	+	+	+	+	+	+
Griseofulvin	—	—	+	—	—	+	—	—	+

[a] + = complete inhibition, and — = no inhibition.

3020 (aromatic), 2940, 2915, 1700 (CO), 1650, 1530, 1490, 1450, 1315, 1110, 1080, 1058, 860, and 748 cm^{-1}; NMR ($CDCl_3$): δ 7.1–7.42 (m, 4H, aromatic), 6.70–7.10 (broad s, 1H, NH), 3.66 (s, 3H, OCH_3), and 2.07 (s, 3H, CH_3) ppm.

Anal.—Calc. for $C_{11}H_{11}NO_3$: C, 64.39; H, 5.37; N, 6.83. Found: C, 64.28; H, 5.41; N, 6.97.

Compounds IV*b*–IV*g* were prepared similarly from III and the appropriate alcohols (Table I).

***p*-Chlorophenyl Benzo[*b*]-3-methyl-2-furancarbamate (IV*h*)**—A solution of III (2.01 g, 0.01 mole) and *p*-chlorophenol (1.28 g, 0.01 mole) in 30 ml of dry benzene was refluxed for 4 hr. The solvent was evaporated, and the residue was crystallized from benzene to give 1.65 g (55%) of the desired compound, mp 155–156°; IR (KBr): 3230 (NH), 1722 (CO), 1670, 1510, 1490, 1460, 1250, 1202, 1100, 1020, 1015, 855, and 752 cm^{-1}; NMR ($CDCl_3$): δ 7.7–7 (m, 8H, aromatic), 7–6.7 (broad s, 1H, NH), and 2.17 (s, 3H, CH_3) ppm.

Anal.—Calc. for $C_{16}H_{12}ClNO_3$: C, 63.68; H, 3.98; N, 4.64. Found: C, 63.79; H, 3.82; N, 4.73.

Compounds IV*i*–IV*n* were prepared similarly.

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Simultaneous Analysis of Hydrocortisone and Hydrocortisone Phosphate by High-Pressure Liquid Chromatography: Reversed-Phase, Ion-Pairing Approach

J. KORPI [*], D. P. WITTMER [*], B. J. SANDMANN [‡], and W. G. HANEY, Jr. [‡x]

Abstract □ The reversed-phase, ion-pairing approach to high-pressure liquid chromatography was applied to the simultaneous analysis of hydrocortisone and its phosphate ester in laboratory-prepared samples and injectable solutions. Results of this technique were evaluated and compared with results of the official procedure.

Keyphrases □ Hydrocortisone—base and phosphate, simultaneous high-pressure liquid chromatographic analysis, prepared samples and injectable solutions □ High-pressure liquid chromatography—reversed-phase, ion-pairing approach, simultaneous analysis, hydrocortisone base and phosphate, prepared samples and injectable solutions □ Ion-pairing—application to high-pressure liquid chromatography, simultaneous analysis of hydrocortisone base and phosphate □ Glucocorticoids—hydrocortisone base and phosphate, simultaneous high-pressure liquid chromatographic analysis

For some time, these laboratories have been interested in the chromatographic applications of ion-pairing (1), and this interest has resulted in a unique approach to the rational separation of ionic compounds by high-pressure liquid chromatography (HPLC) (2). The technique involves the use of a lipophilic stationary phase and the addition of selected ionic compounds to the mobile phase. Ionic analytes injected into this chromatographic system are retained, apparently as a function of the lipophilicity of the ion-pair formed within the system. Therefore, the technique may be referred to as resulting from a reversed-phase, ion-pairing approach to HPLC.

The advantages of this technique over conventional ion-exchange HPLC were discussed previously, and the utility of the approach to the simultaneous analysis of several ionic substances was explored (2). In this investigation, the reversed-phase, ion-pairing approach was applied to the simultaneous analysis of nonionic and ionic compounds, as exemplified by hydrocortisone and hydrocortisone phosphate. These drugs were selected because they may be encountered together in commercial preparations (*e.g.*, injectable solutions) of the phosphate ester. Since free hydrocortisone is regarded as an impurity in these preparations and limited in concentration to less than 1%, procedures for the analysis of hydrocortisone and its ester are required.

Currently (3), the determination of hydrocortisone in the drug substance requires a number of manipulative steps prior to analysis and subsequent use of the enzyme alkaline phosphatase. Problems in the use of the enzyme were noted previously (4), and the complex workup makes the assay lengthy and the results subject to variation. Present methods for the analysis of hydrocortisone phosphate in injectable solutions (5) make no attempt to quantitate the free hydrocortisone present, so a procedure for the simultaneous analysis of the two drugs is of interest.

EXPERIMENTAL

Apparatus and Operating Conditions—A liquid chromatograph