Synthesis of 5-Substituted 2-Oxazolidinethiones and Their Antagonism to Uterotropic Effect of Diethylstilbestrol

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Abstract □ 5-(4-Aminophenoxymethyl)-2-oxazolidinethiones were synthesized by the cyclization of 1-(4-aminophenoxy)-3-amino-2-propanol in the presence of potassium hydroxide and carbon disulfide. This oxazolidinethione, on reaction with suitable isothiocyanates, yielded 5-[4-(substituted thiocarbamido)phenoxymethyl]-2-oxazolidinethiones. These compounds antagonized the uterotropic effects of diethylstilbestrol in female rats and possessed approximate LD_{50} values of 400–>800 mg/kg.

Keyphrases □ 2-Oxazolidinethiones, 5-substituted—synthesized, evaluated for antagonism to diethylstilbestrol effects, rats □ Diethylstilbestrol effects—antagonism by 5-substituted 2-oxazolidinethiones evaluated, rats □ Structure–activity relationships—5-substituted 2-oxazolidinethiones evaluated for antagonism to diethylstilbestrol effects, rats

Significant anti-implantation effects in rats after oral or subcutaneous administration of oxazolidinethiones suggested an antifertility property for these compounds (1). Oxazolidinethiones also lack androgenic and antigonadotropic activity (2). These observations led to the synthesis of substituted 2-oxazolidinethiones and the evaluation of their ability to antagonize the uterotropic effects of diethylstilbestrol in female rats. The various oxazolidinethiones were synthesized according to Scheme I.

EXPERIMENTAL

Melting points were taken in open capillary tubes with a partial immersion thermometer. The compounds were characterized by their sharp melting points and elemental analyses. All compounds were analyzed for carbon, hydrogen, and nitrogen.

Chemistry—*p*-Acetylaminophenol, on reaction with epichlorohydrin, gave 1-(4-acetylaminophenoxy)-2,3-epoxypropane (I). On treatment with succinimide, I yielded 1-(4-acetylaminophenoxy)-2-hydroxypropylsuccinimide (II). Compound II, on hydrolysis, yielded 1-(4-aminophenoxy)-3-amino-2-propanol (III), which cyclized in the presence of carbon disulfide and potassium hydroxide to 5-(4-aminophenoxymethyl)-2-oxazolidinethione (IV). This oxazolidinethione, on reaction with appropriate isothiocyanates, yielded corresponding thiocarbamide derivatives (V–XIII).

1-(4-Acetylaminophenoxy)-2,3-epoxypropane (I)—A mixture of *p*-acetylaminophenol (0.2 mole), epichlorohydrin (0.6 mole), and pyridine (3 ml) was stirred at 100° for 17 hr. The excess epichlorohydrin was distilled off under reduced pressure. The residue was added to a solution of 23 g of sodium hydroxide in 150 ml of water, and the mixture was stirred at room temperature for 24 hr and then extracted with ether. The ethereal extract was washed with water (200 ml), dried over anhydrous calcium chloride, and filtered. Excess ether from the filtrate was removed by distillation under reduced pressure. The solid mass thus obtained was recrystallized from ethanol (70% yield), mp 103–104°.

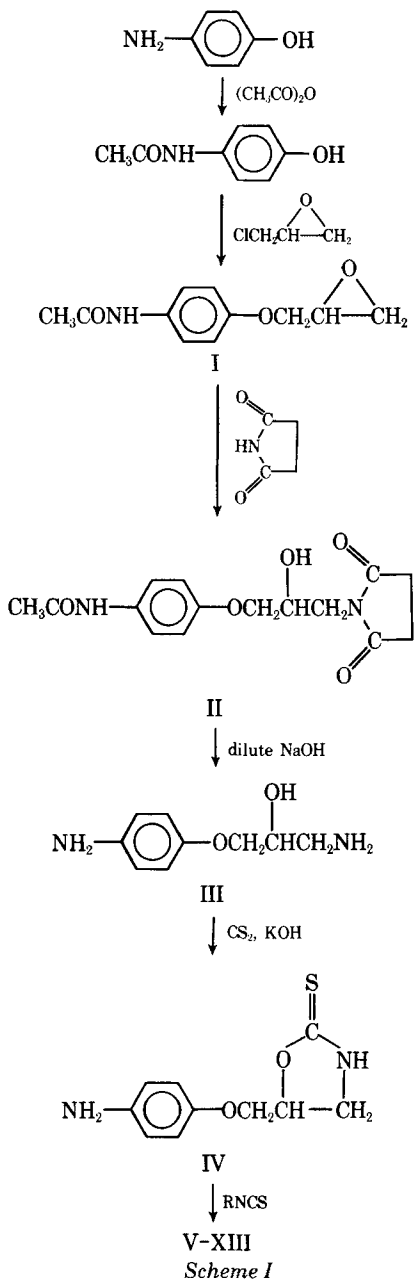
Anal.—Calc. for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.28; N, 6.76. Found: C, 63.97; H, 6.01; N, 6.58.

1-(4-Acetylaminophenoxy)-2-hydroxypropylsuccinimide (II)—A mixture of I (0.1 mole), succinimide (0.1 mole), 100 ml of ethanol, and 4 drops of pyridine was refluxed on a steam bath for 20 hr and then poured over 500 ml of cold water. The separated solid mass was filtered, washed with water, and recrystallized from methanol (80% yield), mp 152–153°.

Anal.—Calc. for $C_{15}H_{18}N_2O_5$: C, 58.82; H, 5.88; N, 9.15. Found: C, 59.12; H, 5.68; N, 8.92.

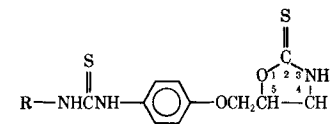
1-(4-Aminophenoxy)-3-amino-2-propanol (III)—A mixture of II (0.1 mole), 33 g of sodium hydroxide, and 120 ml of ethanol was refluxed on a steam bath for 20 hr. Excess ethanol was distilled off under reduced pressure, and 90 ml of cold water was added to the residue. The separated solid mass was collected by filtration, washed thoroughly with water, and recrystallized from ethanol (75% yield), mp 184°.

Anal.—Calc. for $C_9H_{14}N_2O_2$: C, 59.39; H, 7.63; N, 15.38. Found: C, 59.67; H, 7.53; N, 15.00.



Scheme I

Table I—5-Substituted 2-Oxazolidinethiones and Their Antiestrogenic Activity



Compound	R	Melting Point	Yield, %	Formula		Analysis, % Calc.	Found
V	C_6H_5	242°	60	$C_{17}H_{17}N_3O_2S_2$	C	56.82	57.05
					H	4.73	4.62
					N	11.69	11.88
VI	2-$CH_3C_6H_4$	237°	60	$C_{18}H_{19}N_3O_2S_2$	C	57.90	58.29
					H	5.09	5.23
					N	11.26	11.00
VII	3-$CH_3C_6H_4$	248°	65	$C_{18}H_{19}N_3O_2S_2$	C	57.90	58.10
					H	5.09	5.18
					N	11.26	10.99
VIII	4-$CH_3C_6H_4$	244°	50	$C_{18}H_{19}N_3O_2S_2$	C	57.90	58.12
					H	5.09	4.77
					N	11.26	10.91
IX	2-$OCH_3C_6H_4$	241°	55	$C_{18}H_{19}N_3O_3S_2$	C	55.52	55.82
					H	4.88	4.77
					N	10.79	10.53
X	4-$OCH_3C_6H_4$	245°	60	$C_{18}H_{19}N_3O_3S_2$	C	55.52	55.23
					H	4.88	4.54
					N	10.79	11.09
XI	4-ClC_6H_4	95°	65	$C_{17}H_{16}ClN_3O_2S_2$	C	51.83	52.10
					H	4.06	4.04
					N	10.67	10.83
XII	4-BrC_6H_4	258°	80	$C_{17}H_{16}BrN_3O_2S_2$	C	46.57	46.67
					H	3.65	3.72
					N	9.58	9.55
XIII	C_2H_5	245°	85	$C_{13}H_{17}N_3O_2S_2$	C	50.16	50.00
					H	5.48	5.53
					N	13.50	13.29

5-(4-Aminophenoxymethyl)-2-oxazolidinethione (IV)—A cold solution of potassium hydroxide (0.2 mole) in 30 ml of water containing 20 ml of ethanol was added to a mixture of III (0.1 mole) and carbon disulfide (0.1 mole). The mixture was refluxed on a steam bath for 4 hr. Excess ethanol was distilled off under reduced pressure. The residue was diluted with 300 ml of water, cooled in an ice bath, and acidified with 6 *N* hydrochloric acid. The separated solid mass was filtered, washed with water, and recrystallized from acetone (60% yield), mp 230°.

Anal.—Calc. for $C_{10}H_{11}N_2O_2S$: C, 53.57; H, 5.35; N, 12.28. Found: C, 53.89; H, 5.45; N, 12.55.

5-[4-(Substituted Thiocarbamido)phenoxymethyl]-2-oxazolidinethiones (V–XIII)—A mixture of IV (0.01 mole) and the appropriate alkyl or aryl isothiocyanate (0.01 mole) in 40 ml of absolute ethanol was refluxed on a steam bath for 5 hr. Excess ethanol was removed by distillation. The solid mass thus obtained was filtered, washed thoroughly with water and ether, and recrystallized from ethanol. The various substituted 2-oxazolidinethiones (Table I) gave characteristic bands of C=S at 1051–1100 cm^{-1} and >NH at 3300–3340 cm^{-1} in their IR spectra.

Biological Methods—The ability of V–XIII to antagonize the uterotropic effect of diethylstilbestrol was determined using immature healthy female rats (3). The compounds were dissolved in propylene glycol (100%) and administered to 10 rats at a dose of 80 mg/kg ip every 24 hr for 3 days. The control group received an equivalent amount of propylene glycol. Diethylstilbestrol, 2 mg/kg sc, was administered on the 3rd day 6 hr after the last dose of the test compound or propylene glycol.

The decrease in the dry weight of the uterus caused by the test compounds in diethylstilbestrol-treated rats was recorded to evaluate the antiestrogenic activity of the oxazolidinethiones. The results were analyzed statistically using the Student *t* test. The approximate LD_{50} values were determined in mice by intraperitoneal administration of substituted oxazolidinethiones (4).

RESULTS AND DISCUSSION

The ability of V–XIII to antagonize the uterotropic effect of diethylstilbestrol at a dose of 80 mg/kg is recorded in Table II. Diethylstilbestrol treatment significantly increased the dry weight of the uterus to 22.9 ± 2.61 mg as compared to 8.4 ± 1.62 mg in the control rats ($p < 0.001$). All compounds reduced the diethylstilbestrol-induced increase in the dry weight of the uterus of rats. Most of these compounds produced statistically significant reductions in the dry weight of the uterus increased by diethylstilbestrol treatment; maximum reduction was observed with VIII and IX ($p < 0.05$), which possess a methyl or methoxy substituent at the 4-position of the phenyl moiety. Compounds with a 2-methylphenyl (VI) or alkyl (XIII) group showed considerably lower reduction. The introduction of an aromatic nucleus (V and VII–XII) in place of an alkyl substituent (XIII) increased the effectiveness, with the exception of VI where a decrease was observed.

These results failed to provide a definite structure–activity relationship of substituted 2-oxazolidinethiones. The decrease in the uterotropic effects of diethylstilbestrol provided evidence for the greater affinity of substituted 2-oxazolidinethiones without appreciable intrinsic activity toward estrogen receptors in the rat uterus. However, the ability of these compounds to alter the estrogen and progesterone balance, which may lead to the termination of pregnancy, could presumably permit their use as postcoital antifertility agents. Because of the low toxicity, as reflected by their approximate LD_{50} values of 400–>800 mg/kg, further study to

Table II—Antagonism to the Uterotropic Effect of Diethylstilbestrol by 5-Substituted 2-Oxazolidinethiones

Treatment	Weight of Uterus[a], mg	Approximate LD_{50}, mg/kg
Control	8.4 ± 1.62	—
Diethylstilbestrol	22.9 ± 2.61[b]	—
V	10.8 ± 1.20[c]	400
VI	20.0 ± 1.40	>800
VII	12.2 ± 1.42[c]	>800
VIII	8.6 ± 1.28[c]	800
IX	11.4 ± 1.31[c]	600
X	8.5 ± 0.83[c]	600
XI	15.5 ± 1.31	400
XII	14.5 ± 1.18[c]	>800
XIII	16.5 ± 1.00	>800

[a] Screening procedures are as indicated in the text. The values are the mean (± *SE*) of the dry weight of the uteri after administration of diethylstilbestrol in 5-substituted 2-oxazolidinethione-treated rats. [b] Significant difference ($p < 0.001$) from control. [c] Significant difference ($p < 0.05$) from only diethylstilbestrol treated.

determine the antifertility effects of 5-[4-(substituted thiocarbamido)-phenoxymethyl]-2-oxazolidinethiones is warranted.

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Cytotoxic Agents from *Bursera klugii* (Burseraceae) I: Isolation of Sapelins A and B

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Abstract □ A crude chloroform-soluble fraction of the ethanol extract of the leaves of *Bursera klugii* showed activity against two test systems, the P-388 lymphocytic leukemia (3PS) and the human epidermoid carcinoma of the nasopharynx (9KB). The PS activity was due to two constituents, sapelins A and B.

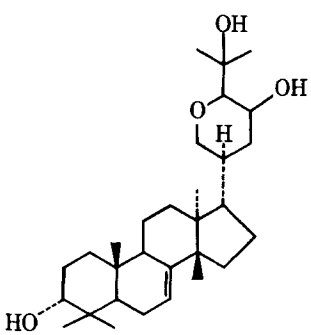
Keyphrases □ Sapelins A and B—isolated from ethanol extract of leaves of *Bursera klugii*, cytotoxic activity evaluated □ *Bursera klugii*—sapelins A and B isolated from ethanol extract of leaves, cytotoxic activity evaluated □ Cytotoxic activity—sapelins A and B evaluated

During a continuing search for plants having tumor inhibitory constituents, the chloroform-soluble fraction of the ethanol extract of the leaves of *Bursera klugii* Macbr (Burseraceae[1]) was shown to be active against the P-388 lymphocytic leukemia test system (3PS) and the human epidermoid carcinoma of the nasopharynx test system[2] (9KB). The activity against the PS test system was due to two constituents of the crude chloroform-soluble fraction.

DISCUSSION

The two major constituents of the chloroform-soluble fraction were triterpenes, sapelin A (I) and sapelin B (II). These compounds were isolated previously from commercial sapele, *Entandrophragma cylindricum* Sprague (1). Isolation of these triterpenes was effected by solvent extraction followed by column chromatography and crystallization. Identification was made by elemental analysis; IR, NMR, and mass spectral data; and direct comparison with an authentic specimen.

Sapelin A demonstrated activities of 130, 136, 127, and 130% test/control (T/C) at 10.0, 5.0, 2.5, and 1.25 mg/kg, respectively. Sapelin B demonstrated activities of 136, 136, 130, and 138% T/C at the same doses, respectively. Activity in the PS system is defined as an increase in the survival of treated animals over that of control animals resulting in a T/C ≥ 125% (2).



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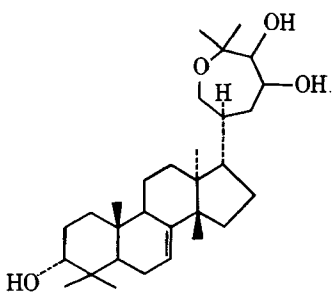

II

EXPERIMENTAL[3]

The leaves of *B. klugii*, collected in Peru during August 1975, were ground and stored at −10° prior to extraction.

The ground material (7.72 kg) was extracted exhaustively in a Lloyd-type extractor with petroleum ether followed by 95% ethanol. The air-dried ethanol extract was subjected to partitioning between chloroform and water. The chloroform phase was first air dried and then vacuum dried. A 300-g portion of the residue was extracted with 5 liters of *n*-hexane in three portions by mechanical stirring. The hexane-insoluble residue was then extracted with 5 liters of ether in three portions using a magnetic stirrer.

The combined ether-soluble fraction, on concentration under vacuum followed by cooling in a freezer overnight, yielded a residue. TLC of this residue showed two spots as major constituents, one corresponding to sapelin A and the other to sapelin B. A 4-g portion of this residue was subjected to alumina (grade III, 160 g) dry column chromatography. Elution with ethyl acetate–benzene (1:1) afforded nearly pure sapelins

[1] Identification was confirmed by Dr. Robert E. Perdue, Medicinal Plant Resources Laboratory, Plant Genetics and Germ Plasm Institute, Beltsville, Md. A reference specimen was deposited in that herbarium.

[2] Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md.

[3] Carbon and hydrogen analyses were carried out by Chemalytics, Inc., Tempe, Ariz. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were run on a Beckman IR-33. NMR and mass spectra were run using a Varian T-60 spectrometer and a Hewlett-Packard quadrupole spectrometer (model 5930), respectively.