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Syntheses and Evaluation of Ketals, Hemithioketals, and Dithioketals of Conjugated Styryl Ketones Principally for Antineoplastic Activity

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Abstract □ Ketals, hemithioketals, and dithioketals of nuclear-substituted styryl ketones were prepared as latentiated forms of the ketones. This undertaking was based on the premise that there is increased acidity in tumors compared to normal tissue, and thus preferential regeneration of the ketone in neoplastic tissue may occur. Attempts to form 1,3-dioxolans of Mannich bases were unsuccessful. The prepared compounds did not possess significant anticancer properties, but analgesic, anti-inflammatory, antihistaminic, and antimicrobial activities were found in the prepared Mannich bases.

Keyphrases □ Antineoplastic activity—ketals, hemithioketals, and dithioketals of conjugated styryl ketones, synthesis and evaluation of activity □ Styryl ketones, conjugated—synthesis of ketals, hemithioketals, and dithioketals, evaluation for antineoplastic activity □ Mannich bases—synthesized from conjugated styryl ketones and acetophenones, evaluation for antineoplastic activity

A recurrent problem in the design of compounds for cancer chemotherapy is the synthesis of derivatives possessing selective toxicity for tumors. Biochemical differences between cancerous and normal cells have been claimed (1), including the increased acidity of certain malignant cells compared to normal tissue (2–4). The increased acidity of tumors has been ascribed to the greater rate of aerobic glycolysis in neoplastic tissue (5), which leads to increased lactic acid production. The pH of a number of tumors has been reported to be ~7.0 (2–4), although such measurements probably recorded the extracellular pH. Therefore, the pH of the intracellular fluid probably is even lower (6), and an average pH value for a number of tumorous tissues has been considered to be ~6.5 (7). Hence, a prodrug permitting the release of a cytotoxic agent under acidic conditions may afford selective lethality of tumors with ameliorated toxicity for normal tissue. Only a few attempts have been made to design compounds based on this pH differential (8–11).

Several investigations in this laboratory involved the preparation of some nuclear-substituted styryl ketones (I) and related Mannich bases (II) for evaluation as anticancer agents (12–14) and in other screens (15–17). While the ketones have not been evaluated against P-388 lymphocytic leukemia, some of the Mannich bases showed promising levels of activity in this screen (12); one compound (IIe) increased the mean survival time in mice by >40% (12). However, murine toxicity was found in II due at least partially to interference with mitochondrial function (18, 19). Therefore, prodrugs of both I and II were prepared, and both I and the prodrugs were evaluated in the P-388 screen so that anticancer activities and murine toxicities of the ketones (I and II) and their latentiated precursors could be compared. Since ketals, hemithioketals, and dithioketals are known to hydrolyze under acidic conditions but are stable in neutral or alkaline media (20), the preparation of these derivatives from I and II may

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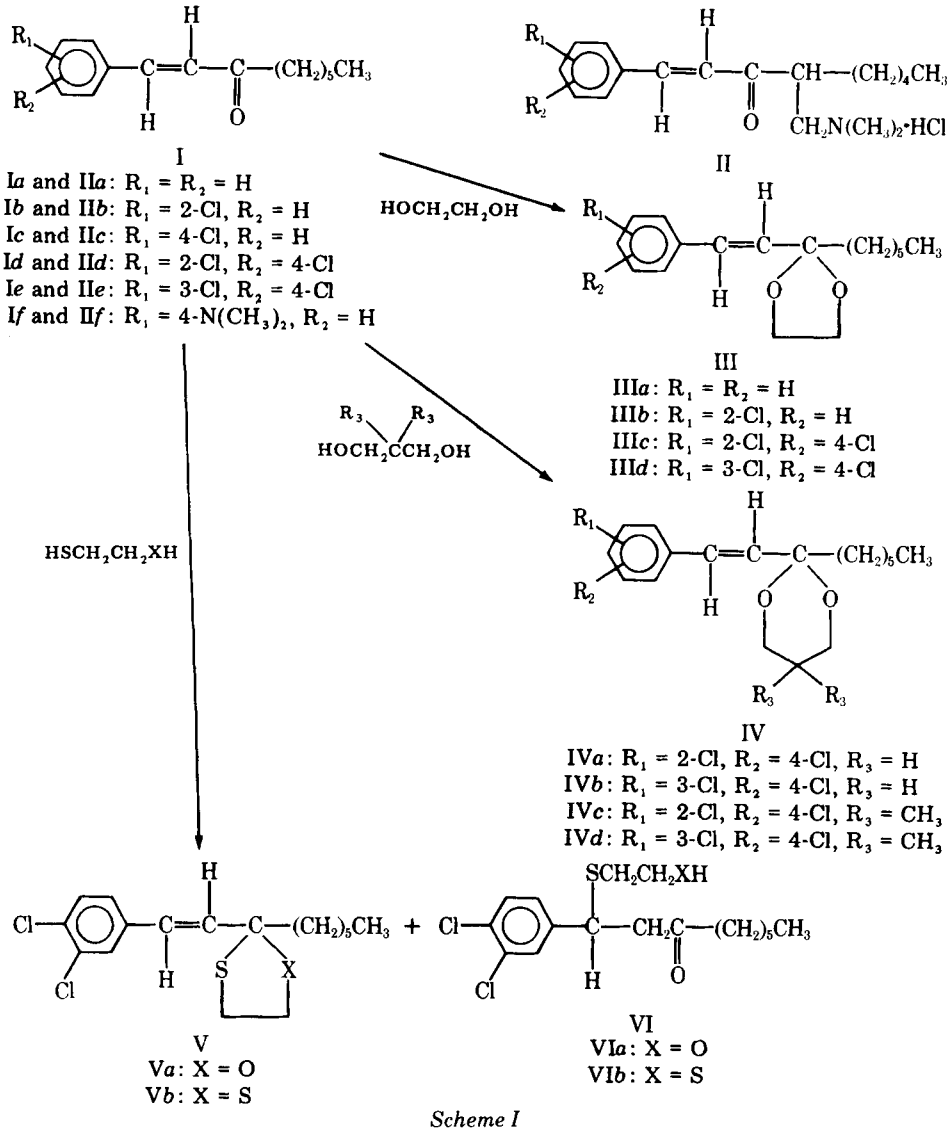


Scheme I

permit selective regeneration of these ketones in neoplastic tissue.

DISCUSSION

A review of synthetic methods available for preparing ketals, hemithioketals, and dithioketals, including the synthesis of these heterocycles derived from α,β-unsaturated ketones (20), outlined various reaction conditions that may be employed.

Initially, several abortive attempts were made to prepare the ketal IIIa from ethylene glycol and 1-phenyl-1-nonen-3-one (Ia) using different catalysts, solvents, and reaction times. However, prolonged heating of the reactants in benzene or toluene using *p*-toluenesulfonic acid as the catalyst led to the successful preparation of III–V, although there occasionally were low yields (Scheme I). The preparation of VII also was undertaken to examine the effect on biological activity of replacing the *n*-hexyl chain by a methyl group. Preliminary attempts to prepare Va and Vb by using milder reaction conditions and piperidine as the catalyst led to the Michael adducts (VIa and VIb) in low yields.

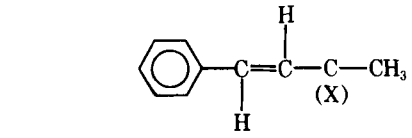
Attempts to react 4-dimethylaminomethyl-1-phenyl-1-nonen-3-one with ethylene glycol to produce the desired ketal were unsuccessful. The only product found on some occasions was the diolefin, 4-methylene-1-phenyl-1-nonen-3-one, which must have originated by the loss of dimethylamine hydrochloride from the Mannich base IIa. To eliminate this deamination process, the Mannich bases IXa and IXb were prepared as shown in Scheme II, but no reaction between IXa and ethylene glycol occurred under the conditions employed.

The antineoplastic evaluation of I and III–IX is shown in Table I. Unless otherwise stated, none of the compounds caused mortalities to mice at a dose of 200 mg/kg. Therefore, the styryl ketones (I) do not have marked murine toxicity, unlike the Mannich bases (II) (12), but only marginal levels of anticancer activity were seen. However, modification of the *n*-hexyl group of Ia led to the analogs VIIa and VIIIa with perceptible beneficial responses in the P-388 lymphocytic leukemia screen.

The lack of antineoplastic activity of the heterocycles III–V and VIIb is not surprising if reversion to the ketones (I) occurs. But the question may be posited as to whether these compounds are stable under neutral or acid conditions and if this is the case, any biological response or lack of activity may be due to the heterocycles *per se*. Seven representative compounds (IIIa, IIId, IVb, IVd, Va, Vb, and VIIa) were hydrolyzed to only a minute extent in buffer solutions of pH 7.0 and 6.4 for 20 hr at 37°; therefore, reversion of these compounds to the precursor ketones may not occur.

Compounds III–IX, with the exception of those indicated under *Experimental*, were evaluated for analgesic, anti-inflammatory, antidepressant, and antihistaminic properties. Of the 15 compounds examined, four met the criterion for analgesic activity, defined as protection of 50% or more in the phenylquinone writhing test (Table I). Compound Va displayed algesic properties. The unsubstituted 1,3-dioxolan (IIIa) showed higher activity than the chlorinated analogs (IIIb–IIId). However, expansion of the dioxolan ring of IIIc and IIId to the 1,3-dioxan heterocycles (IVa and IVb) led to compounds with improved analgesic activities, although insertion of geminal dimethyl groups onto the heterocyclic rings led to virtually inactive compounds, IVc and IVd.

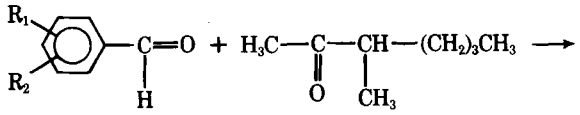
The most active analgesic was the Mannich base IXa; it had an ED_{50} of 20 mg/kg. The analgesic activity of codeine, expressed as an ED_{50} value, is 14.2 mg/kg in the hot plate test (21); although the compounds listed

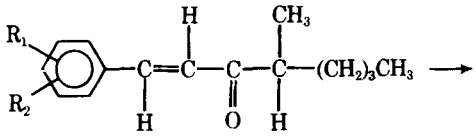


VII

VII*a*: X = =O

VII*b*: X = OCH_2CH_2O

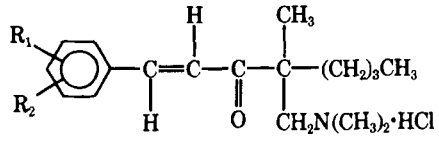




VIII

VIII*a*: $R_1 = R_2 = H$

VIII*b*: R_1 = 3-Cl, R_2 = 4-Cl



IX

IX*a*: $R_1 = R_2 = H$

IX*b*: R_1 = 3-Cl, R_2 = 4-Cl

Scheme II

in Table I were evaluated differently, it appears that IX*a* and codeine have comparable potencies. As noted previously for III, the presence of chlorine atoms in the aromatic ring reduced analgesic potency, and the dichlorinated analog of IX*a*, IX*b*, did not have analgesic activity. It is conceivable that the bulky chlorine atoms hinder alignment of the aromatic ring at a nonpolar area of a receptor.

In certain pathological conditions, an analgesic possessing anti-inflammatory properties can be a useful therapeutic agent. However, III–IX were inactive in the anti-inflammatory screen except for the Mannich base IX*b*, which at a dose of 120 mg/kg showed antagonism to carrageenan-induced rat paw edema of 100 and 17% at 3 and 5 hr, respectively, after treatment[1]. In addition, IX*b* was the only compound that met the criterion for activity[2] in the antidepressant screen, showing antagonism to tetrabenazine-induced ptosis in mice of 50 and 63% at the end of 0.5 and 1 hr, respectively, at a dose of 120 mg/kg. The only other compound with slight activity was IV*c*, which showed antagonism of 54 and 11% at the end of 0.5 and 1 hr, respectively.

All compounds examined in the antihistaminic screen showed activity (Table I), with the two Mannich bases IX*a* and IX*b* showing the greatest potencies. These two derivatives may be regarded as substituted propylamines, which is a class of compounds known to possess antihistaminic activity. Halogenation in the aromatic ring in the antihistaminic propylamines generally enhances activity (22), as was observed with these two compounds.

Some of the series III–IX compounds were examined for antimicrobial activity and were shown to be inactive with the exception of III*a*, which inhibited the growth of *Trichophyton mentagrophytes* at 10 μg/ml, and the Mannich bases IX*a* and IX*b*. Certain Mannich bases display antimicrobial activities (23), and this class of compounds is known to undergo facile deamination (24, 25). Therefore, the antimicrobial activities of Mannich bases may be due to the compound *per se* or to the corresponding deaminated product, *i.e.*, the analogous α,β-unsaturated ketone (26). The Mannich bases IX*a* and IX*b* are incapable of undergoing deamination. Evaluation of simpler Mannich bases (X) in this screen

[1] The reference compound, indomethacin, showed antagonism of 17 and 83% at the end of 3 and 5 hr, respectively, at a dose of 32 mg/kg. Values obtained in excess of 30% are considered to indicate anti-inflammatory activity.

[2] The reference compound was amitriptyline, which showed antagonism at 8 mg/kg of 90 and 49% at the end of 0.5 and 1 hr, respectively. A compound is considered active if it shows an antagonistic effect to tetrabenazine in excess of 50 and 25% after 0.5 and 1 hr, respectively.

Table I—Evaluation of the Styryl Ketones (I) and Related Derivatives (III–IX) for Antineoplastic, Analgesic, and Antihistaminic Activities

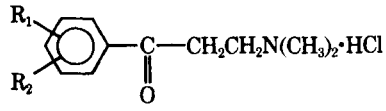
Compound	Maximum Increase in Mean Survival Time[a] (Dose, mg/kg)	Analgesic Activity[b] Dose, mg/kg	Analgesic Activity[b] Percentage Protection	Antihistaminic Activity[c]
I*a*	106 (200)	—	—	—
I*b*	118 (200)	—	—	—
I*c*	100 (400)	—	—	—
I*d*	109 (100)	—	—	—
I*e*	106 (75)	—	—	—
I*f*	97 (200)	—	—	—
III*a*	110 (100)	256	71	15
III*b*	108 (200)	256	44	2
III*c*	110 (100)	256	37	23
III*d*	108 (100)	256	14	3
IV*a*	97 (200)	100	55	15
IV*b*	98 (200)	ED_{50} = 62 mg/kg		19
IV*c*	102 (200)	100	16	16
IV*d*	100 (50)	120	12	28
V*a*	104 (25)	120	Algesic[e]	—
V*b*	113 (50)[d]	—	—	—
VI*a*	104 (200)	—	—	—
VI*b*	102 (50)[f]	120	10	9
VII*a*	126 (400)	—	—	—
VII*b*	102 (200)	120	34	46
VIII*a*	120 (200)	120	23	26
VIII*b*	115 (100)[f]	120	17	20
IX*a*	105 (50)[f]	ED_{50} = 20 mg/kg[g]		50
IX*b*	113 (12.5)[h]	120	1[g]	100

[a] The figures are the ratios of the survival time of treated animals to control animals expressed as a percentage. A compound should increase the mean survival time by 20% to be considered active. [b] Analgesic activity was measured by the percentage protection in the phenylquinone writhing test in mice. Protection in excess of 50% indicates an active compound. [c] The figures indicate the percentage protection of a compound at a concentration of 0.1 mg/ml against a standard dose of histamine. A compound is considered active if 50% or more protection is found. [d] There were 1/6 and 6/6 survivors on Day 5 at dose levels of 200 and 100 mg/kg, respectively. [e] The percentage increase in writhes for this compound was 58%. [f] There were 5/6 and 6/6 survivors on Day 5 at dose levels of 200 and 100 mg/kg, respectively. [g] This compound elicited a Straub tail response. [h] There were 0/6, 1/6, 5/6, and 6/6 survivors on Day 5 at dose levels of 200, 100, 50, and 25 mg/kg, respectively.

where deamination could occur was desired, and it has been claimed that antimicrobial activity is increased as the facility for deamination is enhanced (27). Table II shows that IX and X showed both antibacterial and antifungal activities; while the unsubstituted Mannich bases IX*a* and X*a* had similar activity, the chlorinated compounds in series X showed lower potency than IX*b*.

The levels of analgesic, anti-inflammatory, antidepressant, and antihistaminic activities displayed by IX*a* and IX*b* also were found in X (Table III). All of the series X derivatives showed anti-inflammatory and analgesic properties and, as noted in series IX, X*a*, which has no nuclear halogen, was more potent as an analgesic than were the chlorinated analogs X*b*–X*d*. Series X compounds, like IX, showed antidepressant and antihistaminic activities, but unlike the IX derivatives, the chlorinated compounds, X*b*–X*d*, had lower antihistaminic activity than the unsubstituted compound, X*a*.

In conclusion, this study outlined a synthetic route for the preparation of some potential prodrugs of certain nuclear-substituted styryl ketones and recorded some of the failures to synthesize 1,3-dioxolan derivatives from Mannich bases. The prepared heterocycles did not show improved antineoplastic activity over the precursor α,β-unsaturated ketones, probably due to the inactivity of the compounds *per se* or to the lack of activity of the ketones. Some of the heterocyclic compounds displayed analgesic and antihistaminic activities, although these were of a lower magnitude than some Mannich bases synthesized in this study. While



X

X*a*: $R_1 = R_2 = H$

X*b*: R_1 = 4-Cl, R_2 = H

X*c*: R_1 = 2-Cl, R_2 = 4-Cl

X*d*: R_1 = 3-Cl, R_2 = 4-Cl

Table II—Evaluation of the Mannich Bases (IX and X) for Antimicrobial Activity[a]

Microorganism	IX*a*	IX*b*	X*a*	X*b*	X*c*	X*d*
Escherichia coli (ATCC 8739)	>500	<100	>500	>500	>500	>500
Pseudomonas aeruginosa (ATCC 10145)	>500	>500	>500	>500	>500	>500
Klebsiella pneumoniae (ATCC 4352)	>100	>500	>500	>500	>500	>500
Salmonella typhimurium (G46)	>500	>500	>500	>500	>500	>500
Bordetella bronchiseptica (ATCC 4617)	>500	>500	>500	>500	>500	>500
Staphylococcus aureus (ATCC 6538)	<100	<100	>100	<100	<100	<100
Streptococcus pyogenes (hospital isolate)	>500	<100	>500	<100	>100	<100
Sarcina lutea (ATCC 9341)	>500	<100	>100	<100	<100	<100
Streptococcus faecalis (ATCC 8030)	<100	<100	>500	>500	<100	>100
Bacillus subtilis (ATCC 6633)	>500	<100	>500	>100	<100	<100
Trichophyton mentagrophytes (ATCC 9533)	<100	<10	<10	<10	<10	<100
Microsporum gypseum (ATCC 14683)	<100	<100	>500	<10	<10	<100
Aspergillus niger (ATCC 10535)	>500	<10	>500	>500	>500	>500
Candida albicans (ATCC 10231)	<100	<10	>500	>500	>500	>500
Saccharomyces carlsbergenis (ATCC 9080)	>500	>500	>500	>500	>500	>500
Average antimicrobial activity[b]	183	733	200	467	483	217

[a] The values are the minimum inhibitory concentrations of the compounds in micrograms per milliliter. [b] Figures were calculated from (combined antimicrobial activity × 100)/number of microorganisms in the screen. The combined antimicrobial activity was determined by giving the following scores at the highest potency of the compound against the microorganism: >100 = 2.5, <100 = 5, and <10 = 25.

Table III—Evaluation of the Mannich Bases (IX and X) for Analgesic, Anti-Inflammatory, Antidepressant, and Antihistaminic Activities

	Analgesic Activity[a]		Anti-Inflammatory Activity[b]			Antidepressant Activity[c]			
				Percentage Protection			Percentage Protection		Anti-histaminic
Compound	Dose, mg/kg	Percentage Protection	Dose, mg/kg	3 hr	5 hr	Dose, mg/kg	0.5 hr	1 hr	Activity[d]
IX*a*	ED_{50} = 20 mg/kg[e]		100	17	17	30	25	13	50
IX*b*	120	1[e]	120	100	17	120	50	63	100
X*a*	30	91	120	100	33	60	52	52	83
X*b*	60	91	120	100	87	120	43	48	45
X*c*	120	30	120	100	50	120	33	14	67
X*d*	120	85[e]	120	100	50	120	5	9	43
Reference compound[f]	50	50	32	17	83	8	90	49	100

[a] Analgesic activity was measured by the percentage protection in the phenylquinone writhing test using mice. Protection in excess of 50% indicates an active compound. [b] The anti-inflammatory activity was measured by the percentage protection afforded by the compound to carrageenan-induced edema in rats. Compounds showing greater than 30% protection are considered active. [c] The antidepressant screen measured the antagonism of a compound to tetrabenazine-induced ptosis in mice. A compound is considered active if >50 and >25% protection are obtained at the end of 0.5 and 1 hr, respectively. [d] The antihistaminic screen was undertaken by measuring the effect of the compound (0.1 mg/ml) on a guinea pig ileum preparation. A compound is considered active if 50% protection or more to a standard dose of histamine (0.5 mg/ml) is obtained. The reference compound, diphenhydramine, gives 100% protection at a dose of 0.01 mg/ml. [e] These compounds elicited a Straub tail response. [f] The reference compounds in the analgesic, anti-inflammatory, antidepressant, and antihistaminic screens were aspirin, indomethacin, amitriptyline, and diphenhydramine, respectively.

little antimicrobial activity was found in the nonnitrogenous derivatives, some of the Mannich bases displayed activity against certain pathogenic bacteria and fungi.

EXPERIMENTAL[3]

Melting points and boiling points are uncorrected. Organic extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed using a water aspirator. The boiling point of the petroleum ether was 30–60°. TLC was carried out on all of the compounds, which were purified by column chromatography using aluminum oxide sheets[4] and petroleum ether as the solvent to confirm the homogeneity of the product. The 60-MHz NMR spectra[5] were determined in deuterochloroform with tetramethylsilane as the internal standard. Mass spectra[6] were run at 70 ev, and IR spectra[7] were determined as films.

Syntheses of Compounds—Compounds I*a*–I*f* (28) and II*a* (29) were prepared by literature methods.

*Ketals, Hemithioketals, and Dithioketals (III–V and VII*b)—The general synthetic procedure was as follows. A mixture of the ketone (I) (0.1 mole), the appropriate alcohol or thiol (0.125 mole), and a catalytic quantity of *p*-toluenesulfonic acid (0.1 g) in benzene (200 ml) was heated under reflux for 48 hr. A Dean-Stark trap attached to the apparatus collected the water formed during the reaction. Upon cooling, the mixture was extracted with ether to give viscous yellow oils.

Modifications of this general synthetic route were employed in the preparation of IV, for which toluene was used as the solvent in place of benzene, and for V, for which the length of time of heating under reflux in benzene was reduced to 24 hr. The ketals III and VII*b* derived from ethylene glycol were purified by distillation, while attempts to effect purification in this manner with some of the other compounds (IV and V) invariably led to decomposition to the starting reactants.

The boiling points of III*a*–III*d* and VII*b* were 151°/0.1 mm, 155°/0.15 mm, 161°/0.1 mm, 167°/0.1 mm, and 133°/0.1 mm, respectively. Compounds IV and V were purified by column chromatography using neutral aluminum oxide[8] and eluting the compounds with petroleum ether to give viscous yellow oils. For IV*d*, colorless granular crystals, mp 65–67°, were obtained after chromatography. Attempts to use basic aluminum oxide[9] followed by elution with petroleum ether led to the decomposition of the dioxolan on the column.

NMR and IR spectroscopy and mass spectrometry were carried out on III–V and VII*b*, and the spectra obtained were consistent with the proposed structures. The NMR spectroscopy data found for five representative compounds are: III*a*: δ 7.23 (m, 5, C_6H_5), 6.68 (d, 1, C_1H), 6.02 (d, 1, C_2H), 3.85 (s, 4, OC_2H_4O), 2.12–1.08 [m, 10, $(CH_2)_5$], and 0.88 (t, 3, C_9H_3); IV*b*: δ 7.38–6.78 (m, 3, C_6H_3), 6.45 (d, 1, C_1H), 5.95 (d, 1, C_2H), 3.92 (m, 2, $OCH_2CH_2CH_2O$), 3.78 (t, 4, $OCH_2CH_2CH_2O$), 2.35–1.05 [m, 10, $(CH_2)_5$], and 0.88 (t, 3, C_9H_3); IV*d*: δ 7.48–6.91 (m, 3, C_6H_3), 6.48 (d, 1, C_1H), 5.94 (d, 1, C_2H), 3.48 [q, 4, $OCH_2C(CH_3)_2CH_2O$], 2.04–1.04 [m, 10, $(CH_2)_5$], 1.21 (s, 3, $OCH_2CH_3(-C-)CH_3-CH_2O$), 0.88 (t, 3, C_9H_3), and 0.68 (s, 3, $OCH_2CH_3(-C-)CH_3-CH_2O$); V*a*: δ 7.51–6.91 (m, 3, C_6H_3), 6.44 (d, 1, C_1H), 6.04 (d, 1, C_2H), 4.11 (octet, 2, OCH_2CH_2S), 3.01 (t, 2, OCH_2CH_2S), 2.21–1.08 [m, 10, $(CH_2)_5$], and 0.88 (t, 3, C_9H_3); V*b*: δ 7.45–6.97 (m, 3, C_6H_3), 6.60 (d, 1, C_1H), 6.20 (d, 1, C_2H), 3.25 (s, 4, SCH_2CH_2S), 2.27–1.05 [m, 10, $(CH_2)_5$], and 0.90 (t, 3, C_9H_3). The coupling constant for the olefinic protons in these five compounds was 16 Hz.

[3] Elemental analyses were carried out by Mr. R. E. Teed, Department of Chemistry and Chemical Engineering, University of Saskatchewan.
[4] Eastman-Kodak Co.
[5] Varian T 60 spectrophotometer, Varian Associates of Canada Ltd.
[6] AEI MS-12 mass spectrometer, Picker X-Ray Engineering Ltd.
[7] Beckman IR8 spectrophotometer, Beckman Instruments Inc.
[8] Neutral aluminum oxide (Brockman activity 1.0, 80–200 mesh), Fisher Scientific Co.
[9] Alcoa Chemicals aluminum oxide (F-20), Aluminum Company of Canada.

IR spectroscopy of the ketals III and IV showed loss of the α,β-unsaturated carbonyl peak of I in the region of 1700–1600 cm^{-1}, while the strong absorption at ~965 cm^{-1} due to the out-of-plane methine deformation band of the olefinic group (30) was present. Peaks at ~1190 (w), 1170 (s), and 1095 (w) cm^{-1} were assigned to the carbon–oxygen stretching vibrations associated with the ketal group (31). In the sulfur analogs (V), the absence of carbonyl absorption was noted and the peak at 965 cm^{-1}, while retained, was of diminished intensity compared to III and IV. The sulfur–carbon absorption in the region of 700–590 cm^{-1} (32) was not observed.

Examination of the mass spectrum of a representative ketal (IV*a*) showed that loss of the *n*-hexyl group occurred, producing an ion (A) at *m/e* 175 that was derived from the molecular ion (*m/e* 260). Ion A lost 44 mass units and yielded an ion (B) at *m/e* 131, whose structure was considered to be $C_6H_5CH{=}CHC{\equiv}O^+$. Ion B lost carbon monoxide to form the styrenoid ion $C_6H_5CH{=}C^+H$. Metastable peaks were observed, which supported this fragmentation pathway, and all of the ketals fragmented to give analogous ions. The analytical data and the yields of these compounds are summarized in Table IV.

Initial attempts to prepare the ketal III*a* were either unsuccessful or unsatisfactory. When the reactants were heated in benzene for 4 or 8 hr, little or no reaction product was obtained. When either stannic chloride or boron trifluoride etherate replaced *p*-toluenesulfonic acid (heating under reflux for 48 hr in benzene), no product was obtained. When the reactants were heated under reflux for 48 hr in methanol or chloroform in place of benzene, no reaction occurred, even if anhydrous magnesium sulfate or anhydrous sodium sulfate was added to the reaction mixture. When toluene replaced benzene as the solvent, III*a* was formed in greater yield than when benzene was used as the solvent, but there was an increase in tar formation.

Adducts (VI)—A mixture of 1-(3,4-dichlorophenyl)-1-nonen-3-one (0.1 mole), 2-mercaptoethanol (0.25 mole), and 2 or 3 drops of piperidine in ethanol (100 ml) was heated at 40° for 24 hr. The solvent was removed to give a pale-yellow oil, which was purified by chromatography using aluminum oxide[8]. Elution with petroleum ether gave 1-(3,4-dichlorophenyl)-1-(2-hydroxyethylthio)-3-nonanone (VI*a*) as a colorless oil in a 13% yield; mass spectrum: *m/e* 362 (M^+).

Anal.—Calc. for $C_{17}H_{24}Cl_2O_2S$: C, 56.20; H, 6.66. Found: C, 55.10; H, 6.57.

The thio analog (VI*b*) was prepared in a similar fashion, except that a colorless solid was obtained after completion of the reaction and removal of the solvent. Recrystallization of the solid from acetone produced 1-(3,4-dichlorophenyl)-1-(2-mercaptoethylthio)-3-nonanone (VI*b*) as fluffy, colorless crystals, mp 84–85°, in an 18% yield.

Anal.—Calc. for $C_{17}H_{24}Cl_2OS_2$: C, 53.82: H, 6.38. Found: C, 54.03; H, 6.43.

Styryl Ketones (VIII) and Related Mannich Bases (IX)—1-Phenyl-4-methyl-1-octen-3-one (VIII*a*) was prepared by the literature method (28) as a pale-yellow oil, bp 132°/0.8 mm, in a 64% yield.

Anal.—Calc. for $C_{15}H_{20}O$: C, 83.28; H, 9.32. Found: C, 83.42; H, 9.53.

1-(3,4-Dichlorophenyl)-4-methyl-1-octen-3-one (VIII*b*) was prepared in a similar fashion as a pale-yellow oil, bp 151–153°/0.75 mm, in a 52% yield; mass spectrum: *m/e* 284 (M^+).

Anal.—Calc. for $C_{15}H_{18}Cl_2O$: C, 63.17; H, 6.36. Found: C, 62.35; H, 6.42.

4-Dimethylaminomethyl-4-methyl-1-phenyl-1-octen-3-one hydrochloride (IX*a*) was prepared from VIII*a* by the literature procedure (29) with crystallization from acetone to give fluffy, colorless crystals, mp 153.5–154°, in a 53% yield.

Anal.—Calc. for $C_{18}H_{28}ClNO$: C, 69.77; H, 9.11; N, 4.52. Found: C, 70.12; H, 9.08; N, 4.58.

The corresponding 3,4-dichloro analog (IX*b*), prepared in a similar fashion from VIII*b*, crystallized from acetone as fluffy, colorless crystals, mp 126.5–127°, in a 56% yield.

Anal.—Calc. for $C_{18}H_{26}Cl_3NO$: C, 57.07; H, 6.92; N, 3.70. Found: C, 56.61; H, 6.94; N, 3.67.

Dioxolan Derivative of 4-Dimethylaminomethyl-1-phenyl-1-nonen-3-one—A mixture of 4-dimethylaminomethyl-1-phenyl-1-nonen-3-one hydrochloride (II*a*, 0.1 mole), ethylene glycol (0.125 mole), and a catalytic quantity of *p*-toluenesulfonic acid in benzene (200 ml) was heated under reflux for 48 hr. Water did not appear to collect in a Dean-Stark trap attached to the reaction vessel but the pH of the benzene in the trap was ~1 after 4 hr of heating. After the solvent was removed, a mixture of equal volumes of water and ether was added to the reaction product.

The organic extract was separated and revealed the presence of a compound whose mass spectral, NMR, IR, and TLC characteristics were identical with those of an authentic specimen of 4-methylene-1-phenyl-1-nonen-3-one (29). The aqueous layer was cooled in ice, made basic with aqueous sodium hydroxide solution (10% w/v), and extracted with ether; the product that was isolated was shown by mass spectrometry and IR and NMR spectroscopy to be an unreacted Mannich base.

Other attempts to prepare this ketal used ratios of the Mannich base hydrochloride (II*a*), ethylene glycol, and solvent similar to those described; unless otherwise stated, the reaction proceeded for 24 hr.

1. A catalytic quantity of anhydrous stannic chloride in dry chloroform was used, and the reaction mixture was kept at 40° overnight, from which only unreacted starting material was isolated.

2. The previous experiment was repeated, except that anhydrous magnesium sulfate was added as a drying agent. No reaction occurred.

3. When the reactants were heated under reflux in benzene with anhydrous stannic chloride, the olefin 4-methylene-1-phenyl-1-nonen-3-one and a viscous oil were found.

4. A mixture of Mannich base hydrochloride (0.1 mole), ethylene glycol (0.125 mole), and anhydrous stannic chloride (0.1 g) was heated under reflux in benzene (20 ml) and produced a tarry oil.

5. The reactants were heated under reflux in chloroform containing a catalytic quantity of anhydrous zinc chloride in the presence of molecular sieves[10], from which only unreacted material was isolated.

6. When the previous reaction was repeated using benzene as the solvent and eliminating the molecular sieves, 4-methylene-1-phenyl-1-nonen-3-one was isolated.

7. When boron trifluoride etherate in dry ether was employed and the reaction mixture was heated overnight at 30°, no reaction occurred.

8. The reactants were heated overnight under reflux in chloroform to which adipic acid has been added as the catalyst. The isolated product was 4-methylene-1-phenyl-1-nonen-3-one.

9. Instead of the hydrochloride salt, the free Mannich base was used; the same results were produced.

Dioxolan Derivative of 4-Dimethylaminomethyl-4-methyl-1-phenyl-1-octen-3-one—A mixture of IX*a* (0.1 mole), ethylene glycol (0.125 mole), and a catalytic quantity of *p*-toluenesulfonic acid in benzene (200 ml) was heated under reflux for 48 hr. The mixture was treated as described previously, and only unreacted materials were isolated.

Mannich Bases (X)—The Mannich bases (X) were prepared by the literature method (29) and gave the required derivatives as fluffy, colorless crystals, which were recrystallized from acetone. 3-Dimethylamino-1-phenyl-1-propanone hydrochloride (X*a*) was prepared in a 41% yield, mp 159–160° [lit. (33) mp 160°]. The *p*-chloro analog (X*b*) was obtained in a 43% yield, mp 171–173° [lit. (34) mp 170°]. Compound X*c* was synthesized in a 15% yield, mp 193–195° [lit. (35) mp 193–195°]. Compound X*d* was prepared in a 22% yield, mp 138–139° [lit. (35) mp 138–139°].

Table IV—Ketals, Hemithioketals, and Dithioketals of Conjugated Styryl Ketones

Compound	Yield, %	Formula		Analysis, % Calc.	Found
III*a*	68	$C_{17}H_{24}O_2$	C	78.46	78.56
			H	9.23	9.23
III*b*	62	$C_{17}H_{23}ClO_2$	C	69.27	69.26
			H	7.81	7.81
III*c*	56	$C_{17}H_{22}Cl_2O_2$	C	62.01	62.07
			H	6.69	6.75
III*d*	58	$C_{17}H_{22}Cl_2O_2$	C	62.01	62.16
			H	6.69	6.79
IV*a*	14	$C_{18}H_{24}Cl_2O_2$	C	62.98	63.02
			H	7.05	6.98
IV*b*	16	$C_{18}H_{24}Cl_2O_2$	C	62.98	63.11
			H	7.05	6.92
IV*c*	17	$C_{20}H_{28}Cl_2O_2$	C	64.69	64.45
			H	7.60	7.65
IV*d*	5	$C_{20}H_{28}Cl_2O_2$	C	64.69	63.92
			H	7.60	7.49
V*a*	11	$C_{17}H_{22}Cl_2OS$	C	59.13	59.05
			H	6.42	6.51
V*b*	6	$C_{17}H_{22}Cl_2S_2$	C	56.50	54.54
			H	6.14	6.19
VII*b*	73	$C_{12}H_{14}O_2$	C	75.76	75.51
			H	7.42	7.43

[10] Molecular sieves (type 4A, grade 514, 8–12 mesh), Fisher Scientific Co.

Hydrolyses of the Ketals (IIIa, IIId, IVb, IVd, Va, Vb, and VIIb)—The acetonitrile–buffer solutions were prepared as follows. A solution of monobasic sodium phosphate monohydrate (9.30 g) in double-distilled water was adjusted to 1000 ml (Solution A), and a solution of dibasic sodium phosphate heptahydrate was prepared by dissolving the salt (17.88 g) in water and diluting to 1000 ml (Solution B). A mixture of acetonitrile (500.0 ml) and Solution A (389.0 ml) was placed in a 1000-ml volumetric flask, and Solution B was added to 1000 ml. The pH of the resultant solution was 7.00. The acetonitrile–buffer solution of pH 6.40 was prepared in a similar manner, except that the amount of Solution A was 464.0 ml.

The appropriate ketal (10^{-3} g) was dissolved in acetonitrile–buffer (100.0 ml) at pH 7.00 and 6.40 and heated in a constant-temperature bath at 37° for 20 hr. At the commencement of the experiment and also at the end of 20 hr, 1.0-ml aliquots were withdrawn and diluted to 10.0 ml with the same solvent. The UV spectrum[11] was observed at the λ_{max} of both the ketal and the corresponding ketone. The percentage hydrolysis was obtained by noting the ketone absorption (A), and since there is some end-absorption of the ketal in the region of the λ_{max} of the ketone, the extent of the hydrolyses was obtained from:

$$\% \text{ hydrolysis} = \frac{A_{obs} - A_{\text{end-absorption of ketal}}}{A_{\text{pure ketone}}} \qquad \text{(Eq. 1)}$$

The percentage hydrolyses of IIIa, IIId, IVb, IVd, Va, Vb, and VIIb at pH 7.00 were 3.53, 4.29, 2.23, 1.30, 0.21, 0, and 3.81, respectively. At pH 6.40, the percentage hydrolyses of the same compounds were 1.68, 3.07, 0.13, 0, 0, 0, and 2.75, respectively.

Screening of Compounds—The anticancer screening was carried out by the Drug Research and Development Division of the National Cancer Institute, Bethesda, Md., using their protocols (36). In the P-388 screen, the compounds were administered by the intraperitoneal route into either male or female CD_2F_1 mice, although on rare occasions the $B_6D_2F_1$ strain was employed. The compounds were administered for 9 consecutive days, except for Ia and VIa, for which injections were made every 4 days with a total of two and three injections, respectively. The derivatives were administered in saline (Va, VIb, VIIIb, and IXb), saline with polysorbate 80[12] (Ia–If, IIIa, IVd, VIIa, VIIb, VIIIa, and IXa), saline with alcohol (IVa–IVc), or hydroxypropylcellulose (IIIb–IIId) or as a suspension in saline (VIa). Compound Vb was administered either in saline or in saline containing polysorbate 80.

All of the series III–X compounds, except where noted, were evaluated[13] for analgesic, anti-inflammatory, antidepressant, and antihistaminic activities by previously reported procedures (37). Compounds Vb, VIa, and VIIa were not included in any of these screens; IIIc and Va were not assessed for antidepressant and antihistaminic activities, respectively. The antimicrobial screen[13] was performed by the literature method (37) on the series III–IX compounds with the exception of IVa and VIIa.

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[11] Model 250 spectrophotometer, Gilford Instrument Laboratories.

[12] Tween 80, Atlas Chemical Industries.

[13] Bio-Research Laboratories Ltd., Montreal, Quebec, Canada. It is recognized that the evaluation of the series X compounds against different microorganisms has been discussed in the literature and that the efficacy of Xa and Xb as analgesics has been assessed. They were included in this work for comparative purposes under identical screening conditions as the novel compounds described herein.

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