Syntheses and Bioactivities of 1-(Hydroxyphenyl)-1-nonen-3-ones and Related Ethers and Esters

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Abstract □ A number of nuclear hydroxy styryl ketones and related compounds were prepared and evaluated for antineoplastic and antimicrobial activities as well as for analgesic, anti-inflammatory, and antianaphylactic properties. The second-order rate constants for the reaction of several esters with hydroxide ion in aqueous dioxane (50% v/v) at 36.9° were determined. The screening results showed that activity against P-388 lymphocytic leukemia was found solely with the ethers and that antimicrobial properties were obtained virtually exclusively with the phenolic derivatives. All compounds showed analgesic properties, except for four that were algesic. While little anti-inflammatory activity was found, several compounds showed some antianaphylaxis.

Keyphrases □ Nuclear hydroxy styryl ketones—syntheses, antineoplastic activity, antimicrobial activity, analgesic activity, anti-inflammatory activity, antianaphylactic activity, structure–activity relationships □ Antineoplastic agents, potential—nuclear hydroxy styryl ketones, syntheses, structure–activity relationships □ Structure–activity relationships—nuclear hydroxy styryl ketones, antineoplastic activity, antimicrobial activity, analgesic activity, anti-inflammatory activity, antianaphylactic activity

A number of α,β-unsaturated ketones have antineoplastic and cytotoxic properties (1, 2). In particular, some nuclear hydroxy chalcones displayed activity against the Ehrlich ascites sarcoma in mice (3), while compounds with hydroxy groups in close proximity to the carbonylene function demonstrated activity against several neoplasms (4–8). Therefore, the preparation of certain nuclear hydroxy styryl ketones (I*a*, I*c*, I*e*, and II*a*) was suggested.

Since the hydroxy groups of phenols are susceptible to metabolic inactivation *in vivo* (9), the concept of latentiation (10) was applied in two ways. First, masking the hydroxy group by etherification would be expected to produce compounds (I*b*, I*d*, I*f*, II*b*, III*a*, and III*b*) stable in alkali but capable of regeneration to phenols under acidic conditions (11). Since the claim has been made that the interstitial fluid pH of certain tumors is lower than the plasma of blood afferent to the tumor (12), selective regeneration of the precursor phenols in tumorous tissue might be possible. Second, the formation of benzoate esters (V and VI) of the phenols, which would be hydrolyzed *in vivo* to the phenol, might clarify the relationship between the hydrolysis rate and antineoplastic activity. In addition, since there appears to be a precarious balance between lipophilicity and hydrophilicity in relation to anticancer activity (13), formation of some water soluble Mannich bases (VIII) from nuclear hydroxy styryl ketones was contemplated.

To pursue the evaluation of styryl ketones and related Mannich bases for pharmacological (14, 15) and antimicrobial (16, 17) activities, the proposed compounds were tested in these screens. Since fungal diseases constitute a widespread problem with cancer patients (18), the emergence of an antifungal antineoplastic drug would be welcome.

DISCUSSION

Syntheses—The hydroxy and alkyloxymethoxy compounds I and II were prepared by reacting the appropriate hydroxybenzaldehyde with an alkyloxymethyl chloride followed by condensation with hexyl methyl ketone to give I*b*, I*d*, I*f*, and II*b*, which were hydrolyzed with formic acid to give nuclear hydroxy styryl ketones I*a*, I*c*, I*e*, and II*a*. While the methoxy derivatives III*a* and III*b* were prepared by direct condensation of the dimethoxybenzaldehydes with hexyl methyl ketone, this conventional Claisen–Schmidt condition yielded only tars when nitrobenzaldehydes were employed in the attempted preparation of III*c* and III*d*; similar problems were encountered in attempting the condensation of γ-butyrolactones with nitrobenzaldehydes (19). The failure of these reactions may be due to the high electrophilicity of the carbonyl carbon atom in nitrobenzaldehydes, which may lead to multiple side reactions, including formation of the corresponding phenols (20, 21).

In the present study, the Knoevenagel reaction was employed to give III*c* and III*d* in yields of 52 and 30%, respectively. To increase the III*d* yield, the heating time under reflux was increased from 4 to 36 hr, but the only product isolated was the bis-substituted compound IX in low yield. Attempted condensation of pentafluorobenzaldehyde with hexyl methyl ketone under Claisen–Schmidt conditions gave unreacted components plus resinous products; difficulties were encountered previously with pentafluorobenzaldehyde in the presence of aqueous alkali and other bases (22). Recourse to the methodology employed in the preparation of III*d* afforded IV as well as piperidinyl tetrafluorobenzaldehyde.

The phenols I*a* and I*e* were acylated in the presence of pyridine to give the corresponding esters V–VII in yields of 59–85%. The Mannich bases VIII*b* and VIII*c* were obtained from the precursor ketones I*d* and I*f* in crystalline form, while VIII*a* could not be induced to crystallize.

Hydrolyses—The alkaline hydrolysis rate of esters V–VII was measured in an attempt to seek a correlation between the lability of the esters with antineoplastic activity. The second-order rate constants for reaction of the esters V and VI with hydroxide ion in aqueous dioxane (50% v/v) at 36.9° are given in Table I; as expected, the sulfonate VII as well as the ethers I*b*, I*d*, and I*f* were refractory to hydrolysis under the conditions employed. To ensure that no Michael addition reaction occurred, III*d* was subjected to the same experimental procedures as the esters and was unaffected.

A Hammett plot for esters V and VI showed ρ values of 2.43 ± 0.08 and 1.84 ± 0.07, respectively. The positive ρ values indicate that the hydrolysis reaction is facilitated by electron-withdrawing substituents. In other words, there is a decrease in repulsive forces between the acyl ring and the carbonyl carbon atom in going from the initial state to the transition state for the formation of the tetrahedral intermediate XI (Scheme I). The higher value for the *ortho* esters indicates that the transition state leading to the intermediate XI occurs further along the reaction coordinate than the transition state leading to the corresponding *para* intermediate. This is in accord with the Hammond postulate (23) since the unsubstituted *ortho* ester V*a* is hydrolyzed at a slower rate than the *para*

Table I—Second-Order Rate Constants (k_2 M^{-1} min^{-1}) for the Hydrolysis of the Benzoate Esters V, VI, and X in Aqueous Dioxane (50% v/v) at 36.9°

Compound	Rate Constant	Compound	Rate Constant
V*a*	19.9 ± 0.10	VI*e*	72.0 ± 1.75
V*b*	91.5 ± 2.41	VI*f*	11.1 ± 0.12
V*c*	1646 ± 38.7	VI*g*	6.47 ± 0.14
VI*a*	25.3 ± 0.48	X*a*	8.46 ± 0.35
VI*b*	77.1 ± 1.14	X*b*	22.6 ± 0.13
VI*c*	542 ± 11.3	X*c*	190 ± 1.08
VI*d*	402 ± 8.54	X*d*	5.49 ± 0.01

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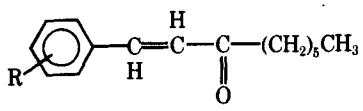
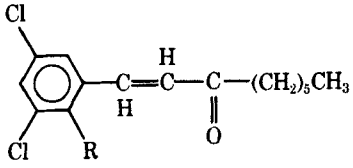
unsubstituted ester VI*a* (Table I). Presumably, the *ortho* ester is less reactive than the *para* ester due to steric impediments to hydroxide-ion attack at the carbonyl function. The ρ value for X was 1.65 ± 0.05, consistent both with the fact that aryl substituents of phenylbenzoates have less effect on the reaction than acyl substituents and with the interpretation given to the different ρ values for V and VI.

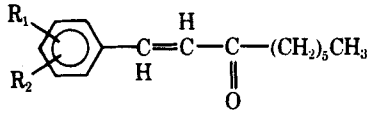
Biological Activities—The antineoplastic evaluation of styryl ketones



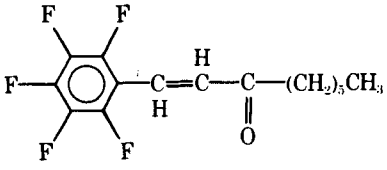
I*a*: R = 2-OH
I*b*: R = 2-OCH_2OCH_3
I*c*: R = 3-OH
I*d*: R = 3-$OCH_2OC_2H_5$
I*e*: R = 4-OH
I*f*: R = 4-OCH_2OCH_3



II*a*: R = OH
II*b*: R = OCH_2OCH_3



III*a*: R_1 = 2-OCH_3, R_2 = 5-OCH_3
III*b*: R_1 = 3-OCH_3, R_2 = 4-OCH_3
III*c*: R_1 = 3-NO_2, R_2 = H
III*d*: R_1 = 4-NO_2, R_2 = H



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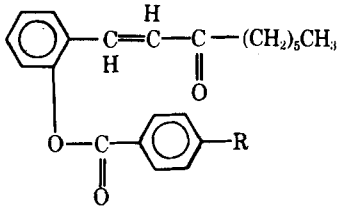
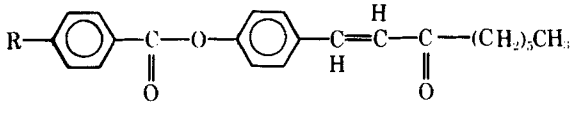
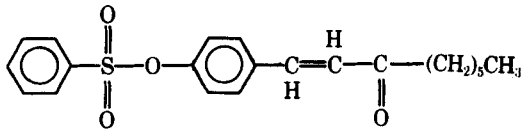


V*a*: R = H
V*b*: R = Cl
V*c*: R = NO_2



VI*a*: R = H
VI*b*: R = Cl
VI*c*: R = NO_2
VI*d*: R = CN
VI*e*: R = Br
VI*f*: R = CH_3
VI*g*: R = OCH_3



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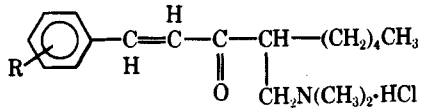
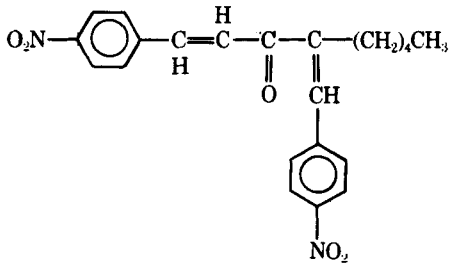


VIII*a*: R = 2-OH
VIII*b*: R = 3-OH
VIII*c*: R = 4-OH



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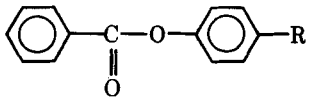
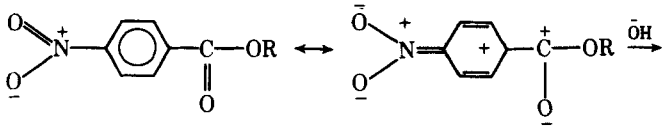
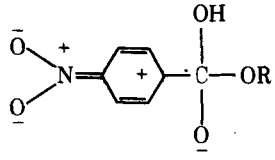


X*a*: R = H
X*b*: R = Cl
X*c*: R = NO_2
X*d*: R = CH_3





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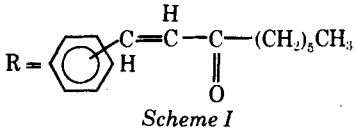

Scheme I

I–VIII is given in Table II. The parent compound, 1-phenyl-1-nonen-3-one, has a maximum T/C of 108%, which is similar to that of the nuclear hydroxy compounds I*a*, I*c*, I*e*, and II*a*, which have an average T/C of 107%. Masking the phenol to give the corresponding ethers I*b*, I*d*, I*f*, and II*b* produced compounds with a perceptible level of activity (an average 20% increase in the mean survival time in mice) unaccompanied by murine toxicity; this result is in contrast to earlier work in which styryl ketones with cytotoxic and antineoplastic properties had significant murine toxicity (16). While the ethers III*a* and III*b* had marginal activity, the nitro compounds III*c* and III*d* were inactive.

In contrast to the ether analogs of the phenols, esters V–VII were bereft

Table II—Evaluation of Styrenoid Ketones I–VIII for Antineoplastic, Analgesic, Anti-Inflammatory, and Antianaphylactic Activities

Compound	Maximum Increase in Mean Survival Time[a], dose in mg/kg	Analgesic Activity[b]		Anti-Inflammatory Activity[c]			Antianaphylactic Activity[d]	
		Dose, mg/kg	Percentage Protection	Dose mg/kg	Percentage Protection 3 hr	Percentage Protection 5 hr	Dose, mg/kg	Percentage Protection
I*a*	112 (200)	256	25	64	0	0	128	8
I*b*	116 (200)	—	—	—	—	—	—	—
I*c*	104 (100)	128	40	128	0	0	25	20
I*d*	125 (100)[e]	128	36	128	0	0	100	45
I*e*	105 (200)	256	12	64	0	−17	8	50
I*f*	121 (100)[f]	128	54	64	−17	0	128	67
II*a*	105 (50)	120	22	120	−34	−34	100	0
II*b*	116 (100)	120	48	120	−6	−17	100	0
III*a*	117 (50)	256	30	128	0	0	128	0
III*b*	115 (200)	128	Algesic[g]	128	17	0	100	20
III*c*	95 (100)	128	Algesic[g]	128	34	8	100	33
III*d*	99 (100)	256	36	128	0	0	128	78
V*b*	101 (25)	256	56	64	−50	0	—	—
V*c*	99 (100)	256	34	64	0	0	128	58
VI*a*	110 (200)	128	Algesic[g]	64	−17	0	128	36
VI*b*	97 (6.25)	128	44	64	0	0	4	0
VI*c*	105 (12.5)	128	59	64	−17	0	128	58
VI*d*	101 (100)	256	41	128	17	66	64	78
VI*e*	104 (12.5)	256	34	128	0	0	128	9
VI*f*	95 (50)	256	36	128	0	0	128	0
VI*g*	105 (12.5)	256	41	128	0	0	128	56
VII	105 (50)	128	Algesic[g]	128	0	0	100	59
VIII*b*	106 (50)[h]	64	90	128	64	100	16	0
VIII*c*	113 (100)[i]	64	96[j]	64	34	17	16	42

[a] The figures are the ratios of the survival time of treated animals to control animals expressed as a percentage. The figures for I*a*, I*b*, I*e*, I*f*, V*c*, VI*a*–VI*c*, and VIII*c* are taken from Ref. 25 and reproduced with permission of the copyright owner. [b] Analgesic activity was measured by the percentage protection in the phenylquinone writhing test. Under these conditions, aspirin gives 50% protection at 52 mg/kg. A compound displaying an effect greater than 50% is considered to be active. [c] The anti-inflammatory activity was measured by the percentage protection afforded by the compound to carrageenan-induced edema. The reference compound, indomethacin, gave 50% protection at 12 mg/kg under these conditions. Compounds showing greater than a 50% protection are considered active. Figures prefaced with a negative sign indicate the percentage increase in the size of edema volume. [d] The antianaphylactic screen measured the potential of a compound to inhibit passive cutaneous anaphylaxis in rats. The reference compound, disodium chromoglycate, reduced the wheal area by 90% at a dose of 100 mg/kg. A compound is considered active if the wheal area is reduced by 50%. [e] An increase of 30% in the lifespan of $B_6D_2F_1$ mice with B16 melanocarcinoma at 50 mg/kg was noted; one in 10 mice was cured. [f] There were 5/6 survivors on Day 5 at dose levels of 200 and 100 mg/kg. [g] The percentage increases in writhes for III*b*, III*c*, VI*a*, and VII were 15, 15, 2, and 10, respectively. [h] There were 2/6 and 5/6 survivors on Day 5 at dose levels of 200 and 100 mg/kg, respectively. [i] There was 1/6 survivors on Day 5 at a dose level of 200 mg/kg. [j] This compound elicited a Straub tail response (35).

Table III—Evaluation of the Nuclear Hydroxy Styryl Ketones and Related Ethers for Antimicrobial Activity[a,b]

Microorganism	I*a*	I*b*	I*c*	I*d*	I*e*	I*f*	II*a*	II*b*	VIII*b*	VIII*c*
Escherichia coli (ATCC 8739)	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
Pseudomonas aeruginosa (ATCC 10145)	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
Klebsiella pneumoniae (ATCC 4352)	>500	>500	<100	>500	>500	>500	>500	>500	>100	>500
Salmonella tymphimurium (G 46)	>100	>500	>500	>500	>500	>500	>500	>500	>500	>500
Bordetella bronchiseptica (ATCC 4617)	>500	>500	>500	>500	>500	>500	>500	>500	>500	>250
Staphylococcus aureus (ATCC 6538)	<100	>500	<100	>500	<100	>500	>250	>500	<100	>250
Streptococcus faecalis (ATCC 8030)	>500	>500	<100	>500	>500	>500	>500	>500	<100	>250
Bacillus subtilis (ATCC 6633)	<100	<100	<100	>500	<100	<100	>500	>500	>100	<100
Trichophyton mentagrophytes (ATCC 9533)	<10	>250	<100	>500	<10	<100	>500	>500	<10	<10
Microsporum gypseum (ATCC 14683)	<10	<100	<100	>500	<10	>100	>500	>500	<10	<100
Aspergillus niger (ATCC 10535)	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
Candida albicans (ATCC 10231)	<100	>250	<100	>500	>500	>500	>500	>500	>10	>100
Saccharomyces uvarum (ATCC 9080)	<10	>500	<100	>500	<10	>100	>500	>500	>10	<10
Average antimicrobial activity[c]	712	92	308	0	654	115	8	0	577	504

[a] The figures in the table are the minimum inhibitory concentrations of the compounds in micrograms per milliliter. [b] The data for I*a*, I*b*, I*e*, and I*f* and most of the results for VIII*c* are taken from Ref. 25 and reproduced with permission of the copyright owner. [c] Figures are calculated from the following expression: (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: >250, 1; >100, 2.5; <100, 5; and <10, 25.

of bioactivity in this screen, with no correlation between the alkaline hydrolysis rate and antineoplastic activity. The Mannich bases VII*b* and VII*c*, while having greater water solubility than I*c* and I*e*, had similar activity against P-388 lymphocytic leukemia and displayed toxicity. Since several biological alkylating agents interfere with thymidylate synthetase and dihydrofolate reductase, which are key enzymes in DNA synthesis, a representative ether, II*b*, was examined and shown to have no effect on either enzyme.

Table II also lists some pharmacological screening data generated on I–VIII. These carbonylenes have either analgesic activity, ranging from 96% protection in the phenylquinone writhing test at a dose of 64 mg/kg in the case of VIII*c* to 12% protection at 256 mg/kg for I*e*, or algesic activity. The utility of an analgesic would be enhanced if anti-inflammatory properties were also present. Compounds V*b* and VIII*b* showed anti-inflammatory properties after 3 hr, and VIII*b* displayed potency at the end of 5 hr. In this series of compounds, antianaphylactic properties were

demonstrated in most compounds, and 36% of the compounds satisfied the criterion for activity; the *para*-cyano ester VI*d* showed the greatest potency.

The compounds listed in Table II were evaluated against 13 species of microorganisms, and the results of the nuclear hydroxy styryl ketones and related ethers are given in Table III. All of the phenolic compounds except II*a* displayed activity; the *ortho*- and *para*-hydroxy derivatives I*a* and I*e* exhibited the greatest potencies. The failure of nuclear halogenation of I*a* to II*a* to increase its antimicrobial activity was surprising since halogenation of phenols is often associated with an increase in antimicrobial activity (24). The most susceptible species to these compounds were *Trichophyton mentagrophytes* and *Microsporum gypseum*. The remaining compounds, III and V–VII, were either inactive at the highest concentration employed (500 μg/ml) or showed only weak activity against a few microorganisms.

EXPERIMENTAL[1]

Melting points and boiling points are uncorrected. Organic extracts were washed several times with water and dried over anhydrous magnesium sulfate. After filtration, the solvents were removed *in vacuo* with a water aspirator. The petroleum ether fraction with a boiling range of 100–120° was used unless otherwise stated. Mass spectra[2] were determined at 70 ev, and the 60-MHz NMR spectra[3] were carried out in deuterochloroform. TLC utilized 20 × 20 cm glass plates, which were pretreated with 0.5 mm silica gel G[4]. The solvent system was chloroform–ethyl acetate–diethylamine (92:5:3). The kinetic studies[5] were carried out with 1-cm quartz cells.

Synthesis of Compounds—Compounds I*a*, I*b*, I*e*, I*f*, V*b*, V*c*, VI*a*–VI*c*, and VIII*c* were prepared using the previously reported methodology (25). The remaining compounds were prepared as follows.

Nuclear Hydroxy and Alkyloxymethoxy Styryl Ketones (I, II, and VIII)—Reaction of *m*-hydroxybenzaldehyde and chloromethyloxyethyl ether produced *m*-ethoxymethyloxybenzaldehyde, bp 82°/0.14 mm, in a 71% yield; NMR spectroscopy was in accord with the proposed structure. The literature method (26) was used, except that the sodium hydride dispersion was added in one operation and the reaction mixture was stirred for 1 hr after the addition of sodium hydride.

3,5-Dichloro-2-methoxymethyloxybenzaldehyde was prepared from 3,5-dichlorosalicylaldehyde and chloromethyloxymethyl ether in a 61% yield in an analogous manner, except that the reaction time was quadrupled. The compound was recrystallized from petroleum ether, mp 90–92°. NMR spectroscopy was in accord with the proposed structure, and the mass spectrum gave peaks at *m/e* 234[6] (M^+, 5%) and 45 (100%).

Treatment of *m*-ethoxymethyloxybenzaldehyde with hexyl methyl ketone in the presence of aqueous sodium hydroxide solution, using the reported method for preparing I*b* and I*f*, gave (*E*)-1-(*m*-ethoxymethyloxyphenyl)-1-nonen-3-one (I*d*) as a yellow oil, bp 168°/0.13 mm, in a 67% yield. TLC indicated that the compound was homogeneous.

Anal.—Calc. for $C_{18}H_{26}O_3$: C, 74.45; H, 9.02. Found: C, 73.96; H, 8.91.

The analogous ketone II*b* was prepared in a similar manner as a yellow oil, bp 187°/0.25 mm, in a 37% yield; mass spectrum: *m/e* 344 (M^+, 0.7%) and 45 (100%). TLC indicated that the compound was chromatographically homogeneous.

Anal.—Calc. for $C_{17}H_{22}Cl_2O_3$: C, 59.14; H, 6.42. Found: C, 60.87; H, 6.31.

(*E*)-1-(*m*-Hydroxyphenyl)-1-nonen-3-one (I*c*) was prepared from I*d* by hydrolysis with aqueous formic acid using the procedure for the formation of I*e* (25). It was crystallized from petroleum ether as a colorless powder, mp 54–56°, in a 73% yield.

Anal.—Calc. for $C_{15}H_{20}O_2$: C, 77.54; H, 8.68. Found: C, 77.57; H, 8.93.

(*E*)-1-(3,5-Dichloro-2-hydroxyphenyl)-1-nonen-3-one (II*a*) was prepared similarly to I*a* (25) and recrystallized from petroleum ether, mp 104–105°, in a 74% yield; mass spectrum: *m/e* 302 (M^+, 6%) and 215 (100%).

Anal.—Calc. for $C_{15}H_{18}Cl_2O_2$: C, 59.81; H, 6.02. Found: C, 59.89; H, 6.23.

The Mannich base VIII*b*, prepared by the reported methodology for synthesizing VIII*c* (25), was recrystallized from acetone to give the desired ketone as a colorless powder, mp 154–156°, in a 38% yield; mass spectrum: *m/e* 238 (M^+ − HCl, 3%) and 58 (100%). TLC showed that the compound was homogeneous.

Anal.—Calc. for $C_{18}H_{28}ClNO_2$: C, 66.34; H, 8.66; N, 4.30. Found: C, 64.11; H, 8.56; N, 4.06.

An attempt to prepare VIII*a* by a similar method to that of VIII*b* (25) produced only a water-soluble blue-green oil; this oil could not be induced to crystallize either by prolonged storage in a vacuum desiccator or by trituration with ether, petroleum ether (bp 60–80°), and benzene. NMR spectroscopy was in accordance with the proposed structure.

Nuclear Methoxy, Nitro, and Fluoro Styryl Ketones (III and IV)—A mixture of 2,5-dimethoxybenzaldehyde (83.09 g, 0.50 mole), hexyl methyl ketone (76.90 g, 0.60 mole), and sodium hydroxide (10.0 g, 0.25 mole) in distilled water (300 ml) was heated under reflux for 12 hr and let stand at room temperature overnight. The two layers were separated, the aqueous layer was washed with benzene (3 × 100 ml), and the organic extracts were combined with the original organic layer and dried. Removal of the solvent and excess hexyl methyl ketone gave a crude product. This product was fractionally distilled to give 2,5-dimethoxybenzaldehyde (16.5 g, 20%), bp 105°/0.3 mm, and (*E*)-1-(2,5-dimethoxyphenyl)-1-nonen-3-one (III*a*) (79.0 g, 58%) as a yellow oil, bp 181°/0.4 mm, which solidified to a wax on standing. The residual tar in the distillation flask was not examined. The mass spectrum of III*a* showed *m/e* 276 (M^+, 44%) and 245 (100%).

Anal.—Calc. for $C_{17}H_{24}O_3$: C, 73.86; H, 8.76. Found: C, 74.00; H, 8.62.

Reaction of 3,4-dimethoxybenzaldehyde with hexyl methyl ketone proceeded in a similar fashion to produce a crude product. This product was fractionally distilled to give (*E*)-1-(3,4-dimethoxyphenyl)-1-nonen-3-one (III*b*) as a yellow oil (58%), bp 168°/0.14 mm, which solidified to a wax on standing, and an orange viscous oil (4 g), bp 183°/0.14 mm. The molecular weight of the orange oil was shown to be 552 by mass spectrometry. The mass spectrum of III*b* showed *m/e* 276 (M^+, 22%) and 191 (100%).

Anal.—Calc. for $C_{17}H_{24}O_3$: C, 73.86; H, 8.76. Found: C, 73.68; H, 8.73.

A solution of *m*-nitrobenzaldehyde (15.0 g, 0.10 mole), hexyl methyl ketone (14.1 g, 0.11 mole), piperidine (8.1 g, 0.10 mole), and acetic acid (6.6 g, 0.11 mole) in dry benzene (100 ml) was heated under reflux with mechanical stirring for 24 hr, cooled, and extracted with water (3 × 100 ml). The organic layer was separated and dried, and removal of the solvent gave a residual brown oil. This oil was fractionally distilled to give *m*-nitrobenzaldehyde (1.2 g, 8%), bp 97°/0.13 mm, mp 55°, and (*E*)-1-(*m*-nitrophenyl)-1-nonen-3-one (III*c*) (13.5 g, 52%), bp 215°/0.10 mm[7], which solidified on standing, yielding yellow flakes. Recrystallization of the flakes from petroleum ether gave III*c* as creamish flakes, mp 50–51°; mass spectrum: *m/e* 261 (M^+, 2%) and 176 (100%).

Anal.—Calc. for $C_{15}H_{19}NO_3$: C, 68.97; H, 7.28; N, 5.36. Found: C, 69.04; H, 7.31; N, 5.38.

The *p*-nitro analog III*d* was prepared as follows. To a vigorously stirred solution of *p*-nitrobenzaldehyde (20.0 g, 0.13 mole) and hexyl methyl ketone (19.0 g, 0.15 mole) in dry benzene (200 ml) were added acetic acid (7.8 g, 0.13 mole) and piperidine (11.1 g, 0.13 mole). The mixture was heated under reflux for 4 hr, using a condenser attached to a Dean–Stark trap, during which time water (3 ml) was collected. The benzene solution was cooled and extracted with water (3 × 100 ml). The organic solvent was removed *in vacuo* to give a black tar to which was added petroleum ether (500 ml); then the mixture was heated under reflux for 2 min.

On cooling, the mixture stood at room temperature for 20 min, and the yellow solution was decanted and evaporated to 200 ml. The solution was stored in a refrigerator overnight (0.05°), and the resultant yellow needles were collected (8.8 g). The original black tar was extracted again with petroleum ether (500 ml) and yielded a further quantity of material (1.7 g). The combined crude products were recrystallized from petroleum ether to give (*E*)-1-(*p*-nitrophenyl)-1-nonen-3-one (III*d*) (10.5 g, 30%) as yellow needles, mp 56–57.5°; mass spectrum: *m/e* 261 (M^+, 14%) and 191 (100%). TLC showed that the compound was homogeneous.

[1] Elemental analyses were carried out by Mr. R. G. Teed, Department of Chemistry and Chemical Engineering, University of Saskatchewan.

[2] AEI MS-12 mass spectrometer, Picker X-Ray Engineering Ltd. Mass spectra were determined by Mr. D. R. Bain, Department of Chemistry and Chemical Engineering, University of Saskatchewan.

[3] T60 spectrophotometer, Varian Associates of Canada Ltd., and WP 60 spectrophotometer, Brucker Spectrospin (Canada) Ltd.

[4] Silica gel N-HR/UV 254 polygram, Fisher Scientific Co. Ltd.

[5] Cary model 118 spectrophotometer.

[6] The molecular ions for compounds containing chlorine atoms refer to the ^{35}Cl-isotope.

[7] On one occasion, the residual black tar exploded violently during the distillation.

Anal.—Calc. for $C_{15}H_{19}NO_3$: C, 68.97; H, 7.28; N, 5.36. Found: C, 69.49; H, 7.43; N, 5.37.

When the time of heating the same quantities of reactants under reflux was extended to 36 hr, a crude product (7.1 g) was obtained. It was recrystallized from petroleum ether to give (*E*)-4-(*p*-nitrobenzylidene)-1-(*p*-nitrophenyl)-1-nonen-3-one (IX) (2.5 g, 5%) as yellow needles, mp 168–170°; mass spectrum: *m/e* 394 (M^+, 25%) and 176 (100%); NMR ($CDCl_3$): δ 8.37–7.26 (m, 11, aromatic H, olefinic H), 2.63–2.49 (m, 2, C_5H), and 1.55–0.79 [m, 9, $(CH_2)_3CH_3$]. TLC indicated a major component, R_f 0.46, with minute traces of three compounds with R_f values of 0.38, 0.51, and 0.58.

Anal.—Calc. for $C_{22}H_{22}N_2O_3$: C, 66.97; H, 5.63; N, 7.11. Found: C, 64.69; H, 5.77; N, 6.70.

The pentafluoro analog IV was prepared in the same manner as III*d*, except that the time of heating under reflux was 1 hr. Distillation of the crude reaction product afforded an oil, bp 108°/1.0 mm, which solidified to a wax and was tentatively identified as 4-piperidinyl-2,3,5,6-tetrafluorobenzaldehyde; mass spectrum: *m/e* 261 (M^+); NMR ($CDCl_3$): δ 10.60–10.30 (d, 1, CHO, J = 2), 3.66–3.00 (m, 6, piperidyl 3′H, 4′H, 5′H), and 2.10–1.54 (m, 4, piperidyl 2′H, 6′H). Continued distillation produced (*E*)-1-(pentafluorophenyl)-1-nonen-3-one (IV) in a 27% yield as a mobile yellow oil, bp 114°/1.0 mm; mass spectrum: *m/e* 306 (M^+, 7%) and 221 (100%); NMR ($CDCl_3$): δ 7.42 (d, 1, C_1H, $J_{1,2}$ = 16.0), 6.92 (d, 1, C_2H, $J_{2,1}$ = 16.0), 2.62 (t, 2, C_4H, $J_{4,5}$ = 7.0), and 2.18–0.63 [m, 11, $(CH_2)_4CH_3$].

Anal.—Calc. for $C_{15}H_{15}F_5O$: C, 58.82; H, 4.94. Found: C, 58.37; H, 4.76.

Esters of Nuclear Hydroxy Styryl Ketones (V–VII)—The method for preparing the esters V and VI was described previously (25). In the case of V*a*, the crude product was a yellow viscous oil, which gave an orange oil, bp 198–206°/0.2 mm, on distillation. TLC of the product indicated two components, R_f 0.64 and 0.18; the component with R_f 0.64 corresponded to the unreacted ketone I*a*. Attempted separation of the reaction components using silica gel column chromatography and the same solvent mixture as employed for TLC failed. The oil turned brown gradually on attempted redistillation, and the distillation was discontinued. NMR spectroscopy and mass spectrometry indicated that the desired ester had been formed.

The syntheses of VI*d*–VI*g* were undertaken successfully, and the esters were recrystallized from petroleum ether.

(*E*)-1-(*p*-Cyanobenzoyloxy)-phenyl-1-nonen-3-one (VI*d*) was prepared in a 72% yield as colorless crystals, mp 103–105°. TLC indicated that the compound was homogeneous.

Anal.—Calc. for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 75.92; H, 6.61; N, 3.59.

The corresponding *p*-bromo ester VI*e* was prepared in an 82% yield as colorless needles, mp 129–130.5°.

Anal.—Calc. for $C_{22}H_{23}BrO_3$: C, 63.62; H, 5.58. Found: C, 63.69; H, 5.55.

The corresponding *p*-methyl ester VI*f* was prepared in a 60% yield as colorless plates, mp 107–108°.

Anal.—Calc. for $C_{23}H_{26}O_3$: C, 78.83; H, 7.48. Found: C, 78.85; H, 7.46.

The corresponding *p*-methoxy ester VI*g* was prepared in a 67% yield as colorless plates, mp 100–101°.

Anal.—Calc. for $C_{23}H_{26}O_4$: C, 75.36; H, 7.15. Found: C, 75.19; H, 7.28.

The sulfonyl ester VII was prepared in a similar manner, except that the reactants were heated together under reflux for 1.5 hr and then stirred mechanically at room temperature for a further 1.5 hr. The crude product obtained by extraction was recrystallized from methanol to give (*E*)-1-(*p*-benzenesulfonyloxy)-phenyl-1-nonen-3-one (VII) as colorless flakes, mp 47–48.5°, in a 76% yield; mass spectrum: *m/e* 372 (M^+, 9%) and 77 (100%).

Anal.—Calc. for $C_{21}H_{24}O_3S$: C, 67.70; H, 6.50. Found: C, 68.03; H, 6.51.

Benzoate Esters (X)—Phenyl benzoate (X*a*) was obtained commercially[8], purified by recrystallization from ethanol, and melted at 69.5–70° [lit. (27) mp 71°]. The esters X*b*–X*d* were prepared by acylation of phenol with the appropriate acid chloride using the method applied to the synthesis of V and VI. The crude esters were recrystallized from petroleum ether to give: X*b*, mp 87–89° [lit. (28) mp 87–87.5°], in a 50% yield; X*c*, mp 142–143.5° [lit. (29) mp 142.5°], in a 64% yield; and X*d*, mp 70–72° [lit. (30) mp 71–72°], in a 39% yield. In addition, the structures of X*b*–X*d* were confirmed by elemental analysis.

[8] BDH Chemicals Ltd.

Kinetic Studies on V–VII and X—The hydrolysis rates of V, VI, and X were determined spectrophotometrically in aqueous 1,4-dioxane (50% v/v) by measuring the optical density (OD) of the appropriate phenoxide ion to completion of the reaction.

A mixture of analytical grade 1,4-dioxane (4 liters) and sodium hydroxide pellets (40 g) was heated under reflux for 18 hr and distilled *via* a 61-cm Vigreux column. The distilled 1,4-dioxane was reheated under reflux with sodium hydroxide pellets, distilled at atmospheric pressure, and stored unexposed to moisture. The wavelengths (max) of the phenoxide ions from I*a*, I*e*, phenol, *p*-chlorophenol, *p*-nitrophenol, and *p*-methylphenol were 406, 389, 299, 301, 405, and 289 nm, respectively. The Beer–Lambert law for the phenoxide ions in aqueous dioxane was confirmed by plotting the optical density *versus* phenoxide concentrations, using five different concentrations of the appropriate phenol to which was added a drop of aqueous sodium hydroxide solution (10 *M*).

The determinations proceeded as follows. Initially, a wavelength of the phenoxide ion was chosen that was least obstructed by absorptions of the corresponding ester. Then the ester was placed in a two-component kinetic flask and dissolved in aqueous 1,4-dioxane (50% v/v, 10 ml) while aqueous sodium hydroxide solution (10 ml) was placed in the side arm flask along with sufficient sodium chloride to maintain an ionic strength of 2.8×10^{-3} *M* of the resultant solution after mixing. The reaction flask was placed in a constant-temperature water bath at 36.9 ± 0.02° for 30 min. The side arm flask was then rotated upwards so that its contents were added to the solution of the ester in the main flask, and the resultant mixture was shaken to ensure thorough mixing and transferred immediately to the thermostated UV cells in the spectrophotometer.

The reaction was followed by recording continually the optical density of the phenoxide ion to infinity at the previously determined wavelength. The time lag between the mixing of the two solutions and the beginning of the recording of the optical density was ~22 sec and was incorporated into the subsequent calculations. The kinetic runs were carried out in duplicate under pseudo-first-order conditions at two different hydroxide concentrations (2.1×10^{-3} and 2.8×10^{-3} *M*), except for the *p*-nitro esters in which case the hydroxide concentrations were 2.1×10^{-3} and 7.0×10^{-4} *M*. The pseudo-first-order constants, k^1, were determined from the expression:

$$k^1 t = \frac{2.303 \log OD\alpha - OD_0}{OD\alpha - OD_t} \qquad \text{(Eq. 1)}$$

in which $OD\alpha$ is the optical density of the solution at infinity, *i.e.*, when 100% of the reaction had occurred, and it was found that the observed $OD\alpha$ = calculated $OD\alpha$ ± 3%. In addition, OD_0 represents the initial optical density and OD_t is the optical density at time *t*. The constant k^1 was calculated by plotting log $OD\alpha - OD_t$ *versus* time. The best fit of the line to the points was obtained by a least-squares treatment of the data using an HP 2000 computer. Table I indicates the second-order rate constants, k^2, which were obtained by dividing the values of k^1 by the sodium hydroxide concentration. For each ester series, log $(k^2R)/(k^2H)$ was plotted *versus* σ_p (31), and the rho values were obtained by computerization. The σ_p value of the 1-nonen-3-onyl group was obtained by plotting the k^2 values for VI*a* and X*a*–X*d* *versus* the Hammett σ_p values of the nuclear substituents (31) for X*a*–X*d*.

No hydrolysis of I*b*, I*d*, I*f*, or VII under these conditions was found using 2.0 *M* sodium hydroxide solutions, and attempted hydrolysis of VI*c* using imidazole (0.1 *M*) was unsuccessful.

Screening of Compounds—For the P-388 anticancer screen[9], the compounds were administered in saline with polysorbate 80[10], except for I*a*, VI*a*–VI*c*, and VIII*a*, which were administered in saline, I*b*, II*a*, and II*b*, which were administered in hydroxypropylcellulose, and I*f*, which was administered in saline with alcohol. The compounds were injected daily for 9 consecutive days by the intraperitoneal route into CD_2F_1 mice, except for I*c* and VIII*b* for which $B_6D_2F_1$ mice were used.

Literature procedures were used in the pharmacological evaluation[11] (33). The antimicrobial evaluation[11] was carried out by the reported methodology (25) using concentrations of 500, 250, 100, 50, and 10 μg/ml. Thus, a designation of >250 means that the compound inhibited growth of the microorganism at 250 μg/ml but not at 500 μg/ml. During the antimicrobial screening, the sample of *Salmonella typhimurium* (ATCC 13311) died and was replaced by a strain designated G46. All of the

[9] The anticancer screening was carried out by the Drug Research and Development Division, National Cancer Institute, Bethesda, Md., using their protocols (32).

[10] Tween 80, Atlas Chemical Industries.

[11] Pharmacological and antimicrobial evaluations were conducted by Bio-Research Laboratories, Montreal, Quebec, Canada.

compounds listed in Table II were screened *versus* the G46 strain of *S. typhimurium*, except for Ia, Ib, Ie, If, Vb, Vc, VIIa–VIIc, and VIIIc, which were assessed against the ATCC 13311 microorganism.

Evaluation of IIb against thymidylate synthetase and dihydrofolate reductase was done according to a literature method (34).

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New and Simple Methylene Blue Colorimetric Assay for Glycyrrhizin in Pharmaceuticals

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Abstract ☐ A new colorimetric method for glycyrrhizin in licorice and drug preparations is given. The method is based on coupling the acidic genin, glycyrrhetinic acid, with methylene blue and measuring the extinction of the coupled compound solution in chloroform–alcohol.

Keyphrases ☐ Glycyrrhizin—analysis, methylene blue colorimetry ☐ Antiulcerogenic agents—glycyrrhizin, methylene blue colorimetric analysis ☐ Colorimetry—analysis, glycyrrhizin, methylene blue ☐ Methylene blue—analysis, glycyrrhizin

Licorice, the root and subterranean stem of different varieties of *Glycyrrhiza glabra*, has long been used in medicine. The drug and some of its preparations are official in many pharmacopoeias. Besides being a valuable flavoring and sweetening agent, the drug has demulcent, expectorant, and antispasmodic action. Recently, it was shown to be effective in gastric ulcer treatment and to have a cortisone-like action in rheumatic arthritis and other inflammatory diseases (1–3). These activities are due to the active constituent glycyrrhizin, which is the calcium and potassium salt of glycyrrhizic acid; the latter is the diglucopyranosiduronic acid of the pentacyclic triterpenoid sapogenin, β-glycyrrhetinic acid. Simple derivatives of β-glycyrrhetinic acid such as the disodium salt of carbenoxolone have been used extensively in gastric ulcer treatment (4).

BACKGROUND

Glycyrrhizin has been estimated by gravimetric assays of variable weighing forms (5–7); volumetric assays such as direct titration of glycyrrhizic acid, glycyrrhetinic acid, or their salts (6, 8); colorimetric assays (6, 9–12); and colorimetric and spectrophotometric determination after chromatographic separation (13–18).

The reported glycyrrhizin content of licorice has varied greatly. Some investigators attributed this variation to the different analytical methods

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