Synthesis and Physical Properties of Substituted 4-Dimethylaminomethyl-1-phenyl-1-nonen-3-ones Possessing Antitumor Properties

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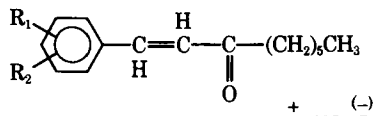
Abstract □ Substituted 4-dimethylaminomethyl-1-phenyl-1-nonen-3-ones were synthesized using the Mannich reaction. The spectral properties and elimination reactions of some prepared compounds were examined. Evidence is presented that the product obtained from the Mannich reaction using 1-(*p*-dimethylaminophenyl)-1-nonen-3-one underwent aromatic rather than alkyl side-chain substitution.

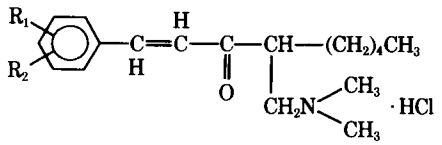
Keyphrases □ 4-Dimethylaminomethyl-1-phenyl-1-nonen-3-ones, substituted—synthesized as possible antitumor agents, physical properties, elimination reactions □ Mannich bases from styryl ketones—synthesis and physical properties of substituted 4-dimethylaminomethyl-1-phenyl-1-nonen-3-ones possessing antitumor properties □ Ketones, unsaturated—synthesis and physical properties of substituted 4-dimethylaminomethyl-1-phenyl-1-nonen-3-ones possessing antitumor properties

In studies of the synthesis of novel antineoplastic agents, it was of interest to prepare a series of nuclear-substituted styryl ketones (I) and the related Mannich bases (II), both groups being α,β-unsaturated ketones. Conjugated olefinic ketones may be regarded as alkylating agents due to their ability to undergo addition reactions with such biologically important nucleophiles as amines and thiols. Chemical studies demonstrated that Michael-type addition reactions of nucleophiles occur with α,β-unsaturated ketones (1, 2). In addition, enzyme-catalyzed alkylation of the thiol group of cysteine with α,β-unsaturated carbonyl compounds has been observed (3, 4). Furthermore, the α,β-olefinic keto group has been implicated as the principal functional group in different compounds possessing antibacterial (5) and antitumor (6–8) activity.

This investigation involved the preparation of the water-soluble Mannich bases from the precursor unsaturated ketones. Mannich bases exhibit various biological responses (9) and have shown activity against certain tumors, including the Ehrlich ascites carcinoma (10), and in the sarcoma 180 screen (11).



I: $R_1 = R_2 = H$, Cl, $N(CH_3)_2$, $\overset{+}{N}(CH_3)_3\overset{(-)}{I}$



II

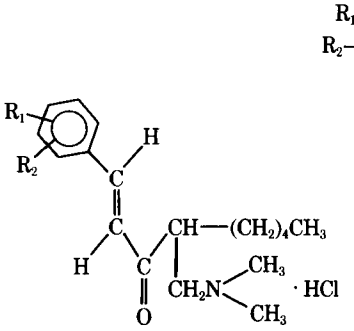
IIa: $R_1 = R_2 = H$
IIb: R_1 = 2-Cl, $R_2 = H$
IIc: R_1 = 4-Cl, $R_2 = H$
IId: R_1 = 2-Cl, R_2 = 6-Cl
IIe: R_1 = 2-Cl, R_2 = 4-Cl
IIf: R_1 = 3-Cl, R_2 = 4-Cl

RESULTS AND DISCUSSION

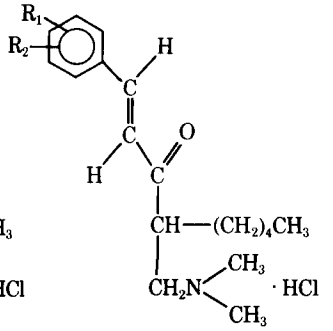
The synthesis and mass spectrometry of the unsaturated ketones (I) were described previously (12). This paper deals with the synthesis, chemical properties, and spectroscopy of the Mannich bases (II). The series of Compounds II was prepared by the Mannich reaction between the unsaturated ketone (I), paraformaldehyde, and dimethylamine hydrochloride in yields ranging from 29 to 43% (Table I). Products other than the desired Mannich base are possible (13, 14) but were not observed in this investigation. It is well known that the choice of solvent (15, 16) and the time of heating under reflux (17, 18) affect the yield of a Mannich base. Table II summarizes the various reaction conditions employed; in general, a 9-hr reflux in ethanol gave the best results.

The Mannich bases, isolated as crystalline hydrochlorides, were stable at room temperature and the structures were confirmed by elemental analysis and spectroscopy. Table III gives some spectroscopic details of these compounds. High-resolution IR spectroscopy has shown that (*E*)-benzylideneacetone (19) and related (*E*)-1-(substituted phenyl)-1-alken-3-ones (20, 21) are capable of existing as s-*cis* (IX) or s-*trans* (X) rotational isomers. The higher frequency band at approximately 1695 cm^{-1} was assigned to the s-*cis* rotational isomer, and the s-*trans*-isomer normally absorbed at 1670 cm^{-1}. In the cases of IIa and IIc, strong carbonyl stretching frequencies were observed at 1655 and 1660 cm^{-1}, respectively, and were identified as due to the s-*trans*-conformation. In addition, both compounds showed weaker absorptions at higher frequencies, denoting the presence of the s-*cis*-isomer. When an *ortho*-chlorine atom was present (IIb, IId, and IIe), only the s-*cis* rotational isomer was seen, and a chlorine atom in the *meta*-position (IIf), shown by UV studies to exert a buttressing effect, gave a spectrum in which only the s-*cis*-isomer was observed.

A strong absorption in the IR spectrum of all of the Mannich bases appeared in the 965–980-cm^{-1} range due to the carbon-hydrogen out-of-plane deformation bands associated with an olefinic bond possessing the *E*-configuration. The NMR spectra indicated doublets at δ 7.70–8.10 and 6.82–6.93 due to the protons at positions 1 and 2, respectively, with olefinic coupling of 16 Hz confirming the *E*-configuration for these compounds. The *N*-methyl protons of the hydrochloride salts resonated as a broad

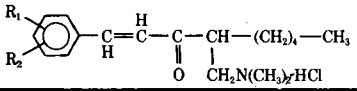


IX: s-*trans*



X: s-*cis*

Table I—Physical Properties of (*E*)-4-Dimethylaminomethyl-1-(substituted phenyl)-1-nonen-3-one Hydrochlorides



Compound	R_1	R_2	Yield, %	Melting Point	Recrystallization Solvent[a]	R_f[b]	Molecular Formula	Analysis, %	
								Calc.	Found
II*a*	H	H	43	138.5–139°	A	0.51	$C_{18}H_{28}ClNO$	C 69.77 H 9.11 N 4.52	69.85 9.38 4.70
II*b*	2-Cl	H	42	95–95.5°	B	0.53	$C_{18}H_{27}Cl_2NO$	C 62.78 H 7.91 N 4.07	62.51 8.05 3.99
II*c*	4-Cl	H	40	139.5–140°	C	0.51	$C_{18}H_{27}Cl_2NO$	C 62.78 H 7.91 N 4.07	62.68 7.95 4.14
II*d*	2-Cl	6-Cl	33	108–108.5°	D	0.52	$C_{18}H_{26}Cl_3NO$	C 57.07 H 6.92 N 3.70	57.25 7.11 3.82
II*e*	2-Cl	4-Cl	35	119–119.5°	C	0.51	$C_{18}H_{26}Cl_3NO$	C 57.07 H 6.92 N 3.70	57.28 6.97 3.67
II*f*	3-Cl	4-Cl	29	111.5°	C	0.50	$C_{18}H_{26}Cl_3NO$	C 57.07 H 6.92 N 3.70	56.84 6.89 3.64

[a] A = ethanol, B = ethyl butyrate, C = acetone, and D = ethyl acetate. [b] On silica gel plates using a mixture of *n*-butanol–acetic acid–water (12:3:5 v/v).

Table II—Reaction Conditions Employed in the Preparation of Mannich Bases II*a*–II*f* from the Corresponding Styryl Ketone, Paraformaldehyde, and Dimethylamine Hydrochloride

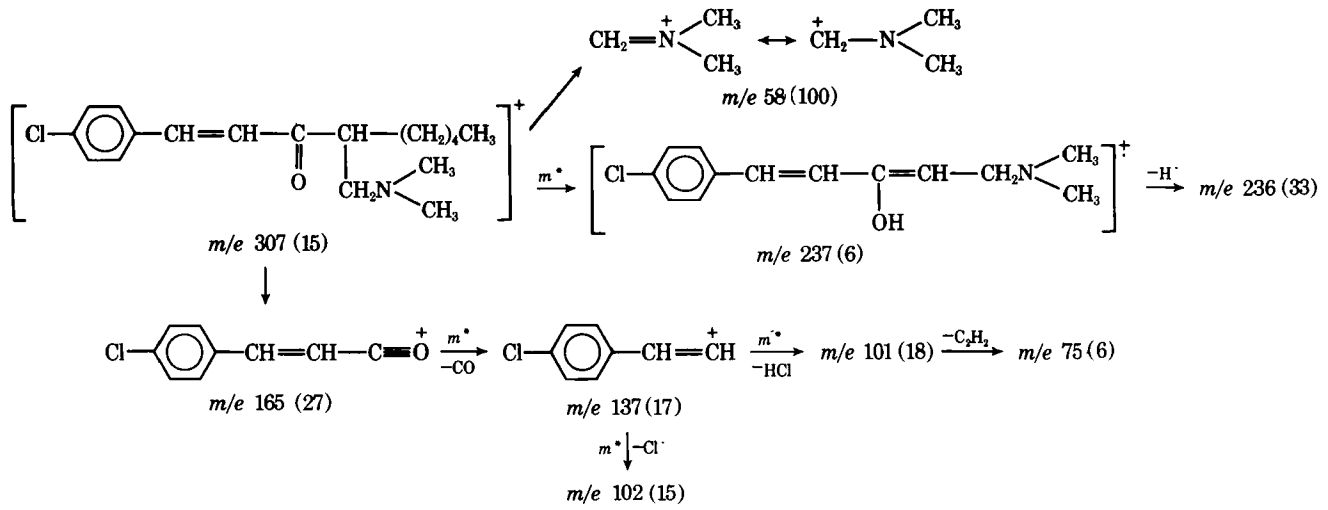
Compound	Styryl Ketone, mole	Paraformaldehyde, mole	Dimethylamine Hydrochloride, mole	Solvent[a] (Volume)	Time of Heating under Reflux, hr	Yield[b], %
II*a*	0.025	0.025	0.025	A (12.5 ml)	9	43
	0.025	0.025	0.025	A (12.5 ml)	21	38
	0.225	0.225	0.225	A (125 ml)	9	45
II*b*	0.025	0.025	0.025	A (12.5 ml)	9	42
II*c*	0.025	0.025	0.025	A (12.5 ml)	9	40
	0.025	0.025	0.025	A (12.5 ml)	21	36
	0.025	0.0375	0.0275	A (4 ml)	2.5	2.5
	0.025	0.025	0.025	I (6.5 ml)	3	13
II*d*	0.025	0.025	0.025	A (12.5 ml)	9	33
II*e*	0.025	0.025	0.025	A (12.5 ml)	9	35
	0.150	0.150	0.150	A (75 ml)	9	34
II*f*	0.025	0.025	0.025	A (12.5 ml)	9	29
	0.025	0.025	0.025	A (4 ml)	3.5	0
	0.025	0.0375[c]	0.025	A (12.5 ml)	6	10
	0.150	0.150	0.150	A (75 ml)	9	45
	0.025	0.025	0.025	I (6.5 ml)	6.5	0

[a] A = ethanol, and I = isoamyl alcohol. [b] Yields based on material obtained, melting within 2° of the analytical sample. [c] Source of formaldehyde was a 37% (w/v) aqueous solution.

Table III—Spectroscopic Properties of Compounds II*a*–II*f*

Compound	IR Spectra[a], cm^{-1}			NMR Spectra[b], δ		Mass Spectra (Relative Intensity)			UV Spectra[c], λ_{max} nm (ε)		
	C=O	C=C	H C=C H	C_1H	C_2H	$M^+ - HCl$	Aryl C_3–C_4 Cleavage Ion	Base Peak			
II*a*	1690 w 1655 s	1630 s	970 s	7.75	6.82	273 (6)	131 (13)	58	299.5 (21,720)	227.5 (8610)	
II*b*	1690 s	1615 s	975 s	8.10	6.90	307 (11)	165 (8)	58	290 (17,770)	230 (9320)	213 (11,650)
II*c*	1690 m 1660 s	1630 s	965 s	7.76	6.83	307 (15)	165 (27)	58	302.5 (24,290)	232 (8860)	228 (9210)
II*d*	1690 s	1615 s	980 s	7.77	6.92	341 (11)	199 (11)	58	273.5 (10,950)	222.5 (13,660)	
II*e*	1690 s	1615 s	975 s	8.05	6.93	341 (10)	199 (14)	58	297.5 (20,420)	235 (10,470)	218.5 (9630)
II*f*	1690 s	1625 s	975 s	7.70	6.84	341 (8)	199 (8)	58	297.5 (24,330)	237 (14,200)	232.5 (14,070)

[a] Determined as potassium bromide disks. [b] Determined in deuteriochloroform (0.5 *M*), $J_{1,2}$ = 16 Hz. [c] Solvent utilized was 95% ethanol.



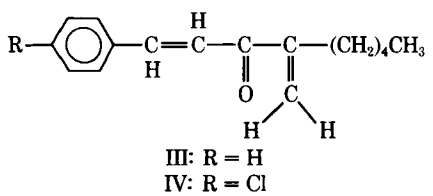
Scheme I—Mass spectral fragmentation pathway of 1-(p-chlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one (IIc)

absorption near δ 2.8. Both IIa and IId appeared as two doublets with a coupling constant of 4 Hz. The remaining Mannich-base salts showed *N*-methyl signals indicating chemical shift nonequivalence, but splitting in each of the two lines was not observed. The *N*-methyl signals of IIa as the free base appeared as a singlet at δ 2.2. Addition of deuterium oxide to the NMR probe showed the disappearance of the low field proton on the charged nitrogen atom and the appearance of the *N*-methyl signals as a singlet. This evidence suggests that coupling of the *N*-methyl protons to the proton of the quadrivalent nitrogen atom occurs, and values of 5–6 Hz for coupling between the proton of a charged nitrogen atom and the protons on the adjacent carbon atom have been observed (22).

The UV spectral data (Table III) indicate that the Mannich bases have absorption maxima in the 273.5–302.5-nm region due to $\pi \rightarrow \pi^*$ transitions of the styryl ketone chromophore (21, 23). Compounds containing an *ortho*-chlorine atom (IIb, IId, and IIe) showed a reduction in the molar absorptivity at the absorption maxima, which is associated with steric hindrance of the aromatic ring with the unsaturated ketone group (24–26). When a *meta*-chlorine atom is present (IIf), the molar absorptivity indicates the existence of a buttressing effect.

The base peak in the mass spectra of all of the Mannich bases was an ion at *m/e* 58, considered to be a reasonance-stabilized species (Scheme I). This ion may be formed by a homolytic bond cleavage process beta to the ion radical site on the nitrogen atom, since this β-cleavage process is known to give prominent peaks in the mass spectra of *N*-alkyl amines (27). The fragmentation patterns[1] for the *para*-chloro Mannich base (IIc) are given in Scheme I. The figures in parentheses after the *m/e* values indicate the relative intensity of the peak compared to the base peak at *m/e* 58 designated 100.

Scheme I shows an ion at *m/e* 237, considered to arise by a McLafferty rearrangement of the γ-hydrogen atom to the carbonyl oxygen. This McLafferty-rearrangement product may lose a hydrogen atom to give the peak at *m/e* 236. An α-cleavage product at *m/e* 165 decomposed with the loss of 28 atomic mass units, corresponding to a loss of carbon monoxide, to give an ion at *m/e* 137 designated as a vinylic cation. This ion at *m/e* 137 lost chlorine and hydrogen chloride ions to give peaks at *m/e* 102 and 101, respectively. It was considered that the ion at *m/e* 101 could lose acetylene to give the ion at *m/e* 75.



III: R = H
IV: R = Cl

In the mass spectra of 1-(*o*-chlorophenyl)-1-nonen-3-ones, a prominent ion corresponding to the loss of chlorine from the molecular ion was observed (12). These peaks were not noted with IIb, IId, and IIe. In addition, the McLafferty-rearrangement products of these compounds did not lose a chlorine atom. It is possible that the loss of *ortho*-chlorine atoms in these Mannich bases may be suppressed due to the favorable fragmentation pathway leading to the ion at *m/e* 58.

TLC showed a mixture of two compounds when an aqueous solution of IIa, as the hydrochloride salt, was neutralized with aqueous sodium hydroxide solution. Resolution of the mixture by column chromatography indicated the presence of IIa as the free base and of the dienone III. The identity of III was elucidated mainly by NMR spectroscopy.

Storage of IIa as the free base at room temperature or by heating the free base at 120° facilitated the formation of III. TLC also indicated that IV was formed when a pure sample of IIc was neutralized with weak bases such as sodium carbonate, ammonium hydroxide, or triethylamine. This elimination mechanism of Mannich bases may partly explain the biological activity of certain members of this class of compounds. The dialkylamino portion could assist transportation of the drug molecule to a site of action and liberate the corresponding unsaturated ketone (28). Furthermore, exchange reactions of Mannich bases with nucleophilic reagents by an elimination–addition mechanism to give sulfides (29) may have possible significance *in vivo*.

It was decided, therefore, to synthesize the eliminated product IV and to compare its antitumor properties to that of IIc. Quaternization of IIc (free base) with methyl iodide gave the corresponding quaternary ammonium compound, which underwent the Hofmann elimination reaction to give IV. A yield of 35% was obtained when benzene–water was used as the solvent mixture, whereas a 75% yield of IV resulted when aqueous alkali alone was employed. The dienone IV was identified mainly by NMR spectroscopy, and the major fragmentation pathways[2] in the mass spectrum are shown in Scheme II.

In contradistinction to the mass spectral fragmentation pathway of the Mannich bases IIa–IIf, an M − 1 peak in the spectrum of IV was observed. In the mass spectra of the unsaturated ketones I, it was shown that, with the exception of the 2,6-dichloro compound, an *ortho*-substituent was lost followed by ring closure to give the benzopyrylium ion (12). The structure of the M − 1 peak at *m/e* 261 is considered to be a substituted benzopyrylium ion. The McLafferty-rearrangement product at *m/e* 192, like the corresponding ion from IIc, had low relative intensity in comparison to the McLafferty-rearrangement ion from 1-(*p*-chlorophenyl)-1-nonen-3-one (V), which had a relative intensity of 42%. Substi-

[1] A complete listing of the mass spectral peaks for Compounds IIa–IIf is available from the authors.

[2] A complete listing of the mass spectral peaks for IV is available from the authors.

tution in the alkyl chain at the α-carbon atom to the carbonyl group, therefore, suppresses the McLafferty-rearrangement process. The α-cleavage ion at m/e 165 in IV, like V, represented the base peak. Decomposition of the base peak into several fragments, confirmed by the appropriate metastable peaks, is shown in Scheme II. In addition, ions at m/e 233 (12), 219 (79), and 205 (16) probably result from M − C_2H_5, M − C_3H_7, and M − C_4H_9 cleavage processes with charge retention on the aromatic fragment.

In an attempt to convert the unsaturated ketone VI to the corresponding Mannich base VII, a yellow solid with a sharp melting point was isolated in low yield. Elemental analysis was in agreement with the proposed structure of VII as the free base, and the mass spectrum gave a prominent peak (60% intensity) at m/e 316 corresponding to the molecular weight of VII. However, NMR spectroscopy showed that in both VI and the product there was a triplet (J = 6.5 Hz) integrating for two protons at δ 2.50 and 2.80, respectively, assigned to the methylene protons at the C-4 position. This would indicate that aminomethylation had not occurred at the predicted position of the alkyl side chain. Sharp *N*-methyl singlets, each integrating for six protons, appeared at δ 2.23 and 3.00. In the δ 6.4–8.0 region, five protons were present; but if the isolated product had Structure VII, then six protons would be expected to appear in this region due to the four aromatic and two olefinic hydrogen atoms. The dimethylaminomethyl group was substituted into the aromatic ring, and Structure VIII was assigned to the product (Scheme III). It was felt that the electron-withdrawing unsaturated keto group would favor substitution in the *meta*-position of the aromatic ring similar to the observation that a Mannich reaction using *p*-hydroxyacetophenone gave substitution of the dialkylaminomethyl group in the *meta*-position of the aromatic ring in contradistinction to substitution of this group in the side chain (30). In addition, the *p*-dimethylamino group would enhance substitution at this position in the aromatic ring.

EXPERIMENTAL[3]

Aminomethylations of (*E*)-1-(Substituted phenyl)-1-nonen-3-ones—The compounds (Table I) were prepared by the following general method. The appropriate α,β-unsaturated ketone (12) (0.025 mole), paraformaldehyde (0.025 mole), and dimethylamine hydrochloride (0.025 mole) in absolute ethanol (12.5 ml) were heated to reflux temperature. Concentrated hydrochloric acid was added until pH 3 was reached, and the mixture was then heated under reflux for 9 hr. The solvent was then removed *in vacuo*, and the residue was dissolved in water (200 ml) and extracted with ether. The ether extracts were washed with water, and the combined aqueous extracts were cooled, basified with aqueous sodium hydroxide solution, and extracted with benzene. Removal of the solvent gave the free Mannich base as a yellow oil. The free base was dissolved in anhydrous ether, the pH was adjusted to 4 with ethanolic hydrochloric acid, and the resultant mixture was cooled to −10°. The precipitate was collected, dried, and recrystallized to yield the appropriate (*E*)-4-dimethylaminomethyl-1-(substituted phenyl)-1-nonen-3-one hydrochloride as colorless prisms (Table I). The effect of yield obtained by varying the experimental conditions is indicated in Table II.

Chromatography of (*E*)-4-Dimethylaminomethyl-1-phenyl-1-nonen-3-one—Neutralization of an aqueous solution of (*E*)-4-dimethylaminomethyl-1-phenyl-1-nonen-3-one hydrochloride, using the general synthetic procedure described earlier, gave an oil which was examined by TLC on silica gel using benzene–acetic acid–95% ethanol (10:1:1 v/v). Two products were present, R_f 0.86 and 0.11. The less mobile major component was identified as (*E*)-4-dimethylaminomethyl-1-phenyl-1-nonen-3-one. IR (film): 2780 s (N—CH_3), 1690 s (C=O), 1660 s (C=O), 1610 s (C=C), 1580 s (aromatic C=C), 1495 m (aromatic C=C), and 980 s [(*E*) HC=CH] cm^{-1}; NMR ($CDCl_3$): δ 7.50 (m, 6, C_1H, C_6H_5), 6.80 (d, 1, $J_{2,1}$ = 16 Hz, C_2H), 3.27–2.00 [m, 9, C_4H, $CH_2N(CH_3)_2$], 1.77–1.02 [m, 8, $(CH_2)_4$], and 0.83 (m, 3, C_9H_3) ppm.

After 3 months at room temperature, 0.25 g of the mixture was chromatographed on silica gel using a 2 × 20-cm column. Elution with petroleum ether (50 ml) and benzene (100 ml) gave a yellow syrup (0.09 g), which was shown by GLC to be 95% of one component and had a R_f value of 0.86 using the TLC conditions described previously. Nitrogen was absent (Lassaigne's test), and spectroscopy indicated the syrup to be essentially (*E*)-4-methylene-1-phenyl-1-nonen-3-one (III). IR (film): N—CH_3 absent, 1670 s (C=O), 1615 s (C=C), 1580 s (aromatic C=C), 1500 m (aromatic C=C), and 980 s [(*E*) CH=CH] cm^{-1}; NMR (carbon tetrachloride): δ 7.33 (m, 7, C_1H, C_2H and C_6H_5), 5.90 (s, 1, C_4CH), 5.64 (s, 1, C_4CH), 2.30 (t, 2, J = 6 Hz, C_5H_2), 1.70–1.07 [m, 6, $(CH_2)_3$], and 0.90 (m, 3, C_9H_3) ppm. A further elution of the column with chloroform (50 ml), acetone–chloroform mixtures (100 ml), and acetone (400 ml) gave an oil (0.1 g), shown by TLC and NMR to be a mixture of IIa and III. The mixture was heated at 120° for 2 hr, and the NMR spectrum of the product showed strong singlets at 5.90 and 5.64 ppm and very small absorptions for C_1H, C_2H, and $N(CH_3)_2$ of the Mannich base IIa.

GLC of the Mannich base and the hydrochloride salt indicated three peaks. TLC of the hydrochloride salt on silica gel, using *n*-butanol–acetic acid–water (12:3:5 v/v) as the developing system, gave one spot (Table I). With the benzene–acetic acid–95% ethanol mixture (10:1:1 v/v), the hydrochloride salt remained at the point of application and no spots appeared for a compound with an R_f value of 0.86.

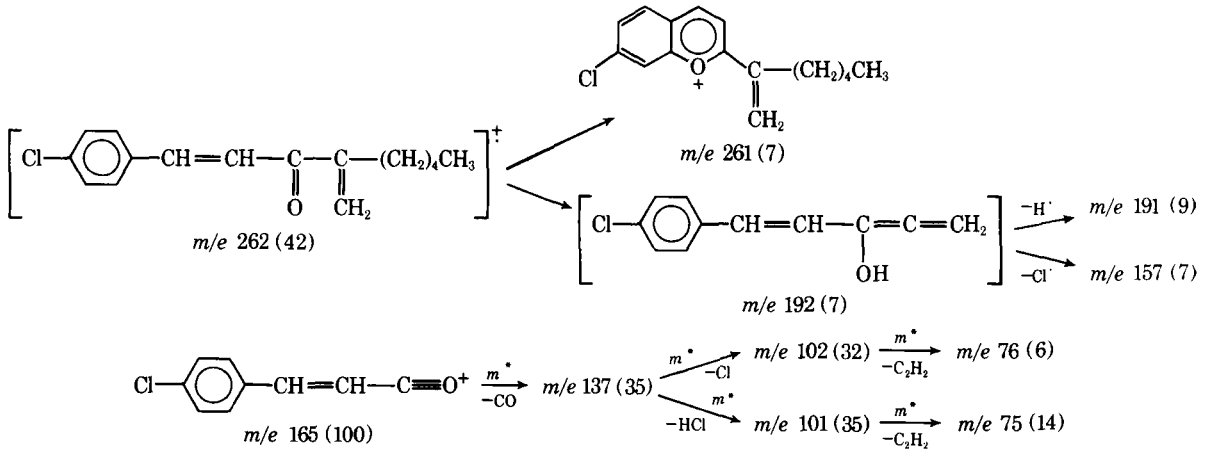
(*E*)-1-(*p*-Chlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one Methiodide—(*E*)-1-(*p*-Chlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one (8.37 g, 0.027 mole), obtained from the hydrochloride by basifying with aqueous sodium hydroxide solution at 0° and extracting with benzene, was dissolved in methyl iodide (7.72 g, 0.054 mole) and ethanol (25 ml) and heated under reflux for 20 hr. The crystals deposited at −10° were recrystallized from ethanol to give the required methiodide (8.43 g, 60%), mp 170° dec., as colorless prisms; IR (KBr): 1690 s (C=O), 1660 s (C=O), 1615 s (C=C), and 970 s (HC=CH) cm^{-1}; NMR ($CDCl_3$, 0.5 *M*): δ 7.87 (m, 3, C_1H and aromatic H), 7.35 (m, 3, C_2H and aromatic H), 4.37–3.93 (m, 2, CH_2N^+), 3.83–3.17 [broad s, 10, C_4H and $^+N(CH_3)_3$], 2.00–1.05 [m, 8, $(CH_2)_4$], and 0.83 (m, 3, C_9H_3) ppm.

Anal.—Calc. for $C_{19}H_{29}ClINO$: C, 50.73; H, 6.50; I, 28.22; N, 3.11. Found: C, 50.89; H, 6.68; I, 28.10; N, 3.17.

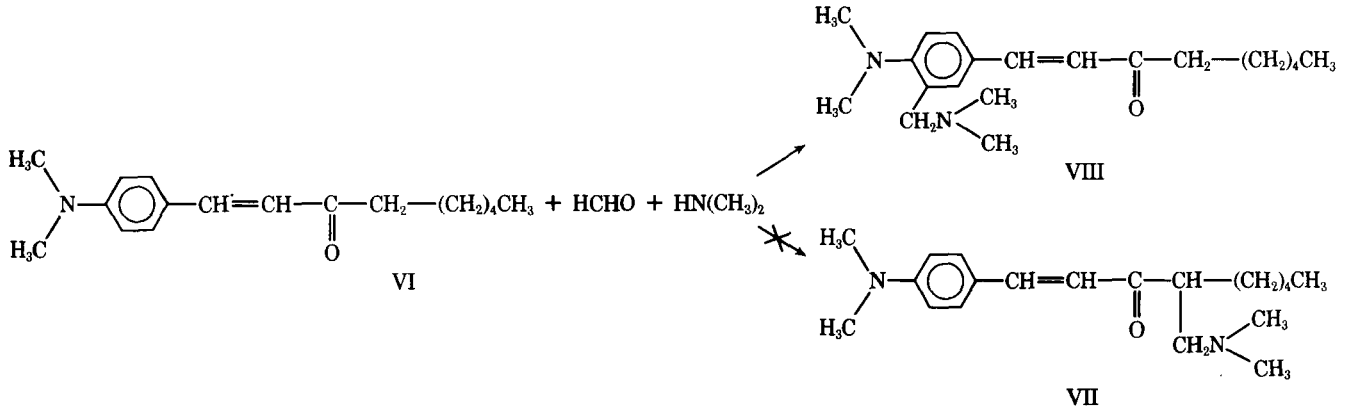
(*E*)-1-(*p*-Chlorophenyl)-4-methylene-1-nonen-3-one (IV)—A mixture of (*E*)-1-(*p*-chlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one methiodide (1.31 g, 0.003 mole), sodium hydroxide (0.30 g, 0.0075 mole), benzene (7 ml), and water (25 ml) was heated under reflux for 1.5 hr. On cooling, the pH of the aqueous phase was adjusted to 6 and the benzene extract was separated. The aqueous solution was extracted with benzene, and the combined organic extracts were removed to give a solid. This solid was recrystallized from ethanol to give the dienone IV (0.271 g, 35%) as off-white prisms, mp 64–65°; UV_{max} (95% ethanol): 228 (ϵ 12,560) and 308 (ϵ 20,970) nm; IR (KBr): 1660 s (C=O), 1600 s (C=C), and 985 s (HC=CH) cm^{-1}; NMR (carbon tetrachloride, 0.5 *M*): δ 7.30 (m, 6, C_1H, C_2H, and aromatic H), 5.90 (s, 1, C_4CH), 5.67 (s, 1, C_4CH), 2.33 (t, 2, J = 6 Hz, C_5H_2), 1.73–1.10 [m, 6, $(CH_2)_3$], and 0.87 (m, 3, C_9H_3) ppm; mass spectrum: 262 (M^+,

[3] Melting points were determined on a Gallenkamp MF-370 apparatus and are uncorrected. The NMR spectra were determined using a Varian T-60 spectrometer with tetramethylsilane as the internal standard. IR absorption spectra were recorded on a Unicam SP-200G spectrophotometer previously calibrated with polystyrene. Band intensities are denoted as s (strong), m (medium), and w (weak). Mass spectra were determined at 70 ev on an AE1 MS-12 single-focusing mass spectrometer operated by Mr. D. Bain, Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. The instrument used a heated inlet system operating near the melting point of the compound, and samples were introduced by the direct probe technique. UV spectra were recorded by Mr. N. Johnson, College of Pharmacy, University of Saskatchewan, Saskatoon, using a Cary model 14 spectrophotometer with 95% ethanol and 1-cm quartz cells. Elemental analyses were performed by Dr. F. B. Strauss, Microanalytical Laboratories, Oxford, England, and by Mr. R. M. Smith, College of Pharmacy, University of Saskatchewan, Saskatoon, using a Coleman model 33 carbon–hydrogen analyzer.

TLC plates, 0.5 mm in thickness, were prepared using silica gel G (E. Merck and Co.) and subsequently heated at 120° for 2 hr prior to use. The chromatograms were developed (20–120 min) and the compounds were detected with a spray composed of a 1% (w/v) aqueous solution of potassium permanganate containing 1% sulfuric acid. Column chromatography was performed using neutral silica gel powder (SilicAR CC-7, 200–325 mesh, Mallinckrodt Chemical Works). GLC was undertaken on a Pye 104 model 64 instrument equipped with flame-ionization detector, using nitrogen as the carrier gas (flow rate of 50 cm^3/min). A 1.52 m (5 ft) × 0.63 cm (0.25 in.) o.d. glass column, containing 4% SE-30 absorbed onto acid-washed and silanized Chromosorb W, 100–200 mesh, was used. The isothermal operating temperature was 220° while the detector temperature was held at 25–30° above the oven temperature during the operation. Organic extracts were washed with water and dried over anhydrous magnesium sulfate.



Scheme II—Mass spectral fragmentation pathway of 1-(p-chlorophenyl)-4-methylene-1-nonen-3-one (IV)



Scheme III—Mannich reaction using 1-(p-dimethylaminophenyl)-1-nonen-3-one

relative intensity 42%).

Anal.—Calc. for $C_{16}H_{19}ClO$: C, 73.13; H, 7.29. Found: C, 73.12; H, 7.10.

In a separate synthesis, a mixture of the methiodide (4.498 g, 0.010 mole), sodium hydroxide (2.80 g, 0.070 mole), and water (70 ml) was heated for 0.5 hr at 70°. Hydrochloric acid was added until the pH was 6, and extraction with benzene afforded a tan solid (2.26 g) which, on recrystallization, gave the pure dienone (1.965 g, 75%).

Mannich Reaction between (*E*)-1-(*p*-Dimethylaminophenyl)-1-nonen-3-one, Paraformaldehyde, and Dimethylamine Hydrochloride—A mixture of (*E*)-1-(*p*-dimethylaminophenyl)-1-nonen-3-one (6.483 g, 0.025 mole), paraformaldehyde (0.751 g, 0.025 mole), dimethylamine hydrochloride (2.039 g, 0.025 mole), and alcohol (25 ml) was heated to reflux temperature, the pH was adjusted to 4, and the mixture was heated under reflux for 9 hr. On cooling, the mixture was stirred at room temperature overnight; removal of the solvent gave a yellow residue which was extracted with benzene. The benzene extract contained unreacted ketone (0.82 g) from melting-point and mixed melting-point evidence. The aqueous extract was cooled in an ice bath, basified with aqueous sodium hydroxide solution, and extracted with benzene to give a yellow solid. TLC on silica gel [*n*-butanol–acetic acid–water (12:3:5 v/v)] suggested two components, R_f 0.50 and 0.84. Recrystallization of the solid from alcohol gave the material with the R_f value of 0.50 (0.14 g, 1.8%). A further recrystallization gave fluffy, yellow needles (0.024 g) identified as 1-(*p*-dimethylamino-*m*-dimethylaminomethylphenyl)-1-nonen-3-one, mp 71.5–72°; IR (KBr): 1650 m (C=O) and 1595 s (C=C) cm^{-1}; NMR ($CDCl_3$): 7.87–7.37 (m, 3), 6.87–6.47 (m, 2), 3.33 (s, 2), 3.00 [s, 6, $N(CH_3)_2$], 2.80 (t, 2, J = 6 Hz), 2.23 [s, 6, $N(CH_3)_2$], and 0.90 (m, 3, C_9H_3) ppm; mass spectrum: 316 (M^+, 60), 134 (100), and 58 (75).

Anal.—Calc. for $C_{20}H_{32}N_2O$: C, 75.59; H, 10.18; N, 8.84. Found: C, 75.52; H, 9.93; N, 8.59.

Attempts to form a crystalline hydrochloride were unsuccessful. Experiments designed to prepare the desired Mannich base (VII) using isoamyl alcohol, acetic acid, or dimethyl sulfoxide as the solvent gave black tars which were not examined.

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Hydrolysis of Steroid Oximes: Mechanism and Products

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Abstract □ The conversion of 17α-acetoxy-6α-methyl-4-pregnen-3,20-dione 3-oxime to the corresponding diketone in acidic media was found to be a first-order reaction at 37°. The effects of incorporating ester groupings at the oxime function or at the C-17 position and modifications in ring B were also investigated. Only the length of the ester chain at the oxime function had a profound effect on the rate constant. From these observations, it is proposed that two competing mechanisms of hydrolysis are involved for the oxime esters.

Keyphrases □ Steroid oximes—hydrolysis mechanisms and products, effects of ester substituents □ 17α-Acetoxy-6α-methyl-4-pregnen-3,20-dione 3-oxime—conversion to diketone, effects of ester substituent at oxime function and C-17 position, modifications in ring B, mechanisms of hydrolysis □ Oximes, steroid—hydrolysis mechanisms and products, effects of ester substituent and ring B modifications □ Hydrolysis—steroid oximes, mechanisms and products

Buhler *et al.* (1) reported that the acid hydrolysis of 11β-hydroxy-11α-methyl-5β-pregnane-3,20-dione dioxime proceeds rapidly to the corresponding 3,20-diketone by the stepwise mechanism shown in Scheme I. The rate of hydrolysis for the 3-oxime could not be measured, but they reported the rate to be 5.5×10^{-4} sec^{-1} for the 20-oxime. The half-life for the overall hydrolysis (Scheme I) was about 21 min in gastric juice.

Several compounds with the 3-oximino function were synthesized in this laboratory (Table I) and reported (2) to be potent progestational agents. The observation of Buhler *et al.* (1) suggested that these oximes might be hydrolyzed rapidly in gastric fluid and that the resulting 3-keto compounds would be responsible for the observed biological activity. Therefore, a study was initiated to measure the kinetic rate of 3-oximino hydrolysis at pH 1.5 and 37° and to determine if other modifications at the oxime function or around the steroid nucleus affected the rate of hydrolysis.

EXPERIMENTAL[1]

Apparatus—A constant-temperature water bath and a spectrophotometer[2] were used.

Buffers—A pH 1.5 buffer, prepared according to USP XVIII (3), was used.

Procedure for Hydrolysis—A stock solution of each compound (Table I) at a concentration of 300 μg/ml was prepared in purified dioxane. A 30-ml aliquot of this solution was further diluted with 20 ml of buffer solution, and then a 5-ml aliquot was placed in each of three 10-ml glass ampuls. The ampuls were sealed and placed in a constant-temperature water bath maintained at 37°. At specified time intervals, the ampuls were removed and each was extracted separately with methylene chloride. The organic layers were transferred to another vial and evaporated, and the residues were saved for their respective assays. The first ampul residue was assayed by the oxime colorimetric method, and the second ampul residue was assayed by the carbonyl colorimetric method. The residue from the third ampul was used for TLC.

Oxime Colorimetric Assay Method—*Sulfanilamide Solution*—Transfer 200 mg of sulfanilamide into a 100-ml volumetric flask, and add approximately 75 ml of distilled water. Heat the flask on a steam bath until solution occurs, and dilute to volume with distilled water.

Iodine–Acetic Acid Solution—Transfer 1.3 g of iodine into a 100-ml volumetric flask, and add approximately 75 ml of acetic acid. Shake the flask until all of the solid dissolves, and dilute to volume with acetic acid.

Sodium Thiosulfate Solution—Transfer 26 g of sodium thiosul-

[1] Some compounds were obtained from the Division of Chemical Research, Ortho Research Foundation, and others were synthesized by published procedures (2).
[2] Shimadzu QV-50.