Synthesis and Evaluation of Some Alkoxy-, Chloro-, and Acyloxy-Conjugated Styryl Ketones Against P-388 Lymphocytic Leukemia and an Examination of the Metabolism and Toxicological Effects of 1-(*m*-Ethoxymethyloxyphenyl)-1-nonen-3-one in Rats

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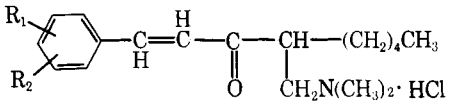
Abstract □ A number of analogs of a new antineoplastic agent, 1-(*m*-ethoxymethyloxyphenyl)-1-nonen-3-one (III*a*) were prepared and evaluated against murine P-388 lymphocytic leukemia. Metabolic studies of III*a* in rats showed that it was sequestered rapidly to the brain and hence probably to other adipose tissue, which may account for the absence of III*a* and metabolites in urine and feces. A detailed toxicological evaluation of III*a* in rats showed marked pathological changes principally in the liver and spleen as a result of erythrophagocytosis from bleeding into the abdomen.

Keyphrases □ Antineoplastics—alkoxy-, chloro-, and acyloxy-conjugated styryl ketones, synthesis and evaluation of activity □ Styryl ketones, conjugated—synthesis of alkoxy-, chloro-, and acyloxy-analogs, evaluation for antineoplastic activity □ 1-(*m*-Ethoxymethyloxyphenyl)-1-nonen-3-one—metabolic and toxicological studies

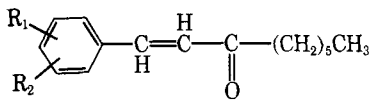
A number of Mannich bases (I) derived from conjugated styryl ketones synthesized in these laboratories displayed activity against P-388 lymphocytic leukemia, but murine toxicity was marked (1, 2). This observation was in contrast to the precursor α,β-unsaturated ketones (II) which, while not causing mortalities under the conditions of evaluation, were bereft of antineoplastic activity (1, 2). More recently, however, a number of conjugated styryl ketones, which were not Mannich bases, showed perceptible, beneficial responses in the P-388 lymphocytic leukemia screen. Thus, ethers III*a–c* increased the median survival time in mice by ~19% at the optimum dose levels, and assessment of III*a* against B-16 melanocarcinoma increased the lifespan by 30% with 1 mouse in 10 being cured (2). Furthermore, the unsubstituted and dichloro styryl ketones (IV*a–d*) also achieved increases in the lifespan of mice with P-388 lymphocytic leukemia of ~20% (3). With the exception of IV*b*, all of the compounds III and IV did not cause mortalities to mice during evaluation at the maximum doses administered (2, 3).

The purpose of the present investigation was twofold: First, molecular modification of the compounds in series III and IV to produce novel derivatives for evaluation in the P-388 screen was planned. Second, a study of the metabolism of III*a* was considered to be a viable undertaking, which may permit the identification of the breakdown products in the body followed by their antineoplastic evaluation and the subsequent design of new compounds on a rational basis.



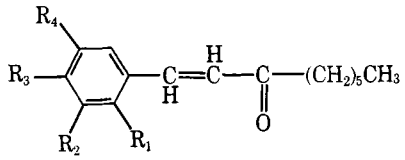
I

$R_1 = R_2 = H, Cl, OH$



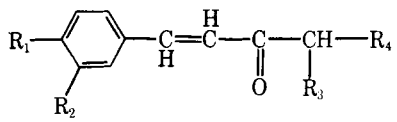
II

$R_1 = R_2 = H, Cl, OH$



III

a: $R_1 = R_3 = R_4 = H$; $R_2 = OCH_2OC_2H_5$
b: $R_1 = R_4 = OCH_3$; $R_2 = R_3 = H$
c: $R_1 = R_4 = H$; $R_2 = R_3 = OCH_3$



IV

a: $R_1 = R_2 = H$; $R_3 = CH_3$; $R_4 = (CH_2)_3CH_3$
b: $R_1 = R_2 = Cl$; $R_3 = CH_3$; $R_4 = (CH_2)_3CH_3$
c: $R_1 = R_2 = R_3 = R_4 = H$
d: $R_1 = R_2 = Cl$; $R_3 = R_4 = H$

RESULTS AND DISCUSSION

The synthetic program was directed toward the preparation of the ethers (V–VII), the ketones (VIII), the esters (IX), and finally some prodrugs of 4-phenyl-but-3-en-2-one, (X and XI). The reasons for proceeding in this direction were as follows. It has been claimed that the pH of a number of tumors is lower than normal tissue (4–6), and the average pH value for many tumors has been estimated to be ~6.5 (7). Therefore, it is conceivable that the levels of antineoplastic activity obtained with III*a*, namely, increases in mean survival times of 25 and 30% in mice bearing P-388 lymphocytic leukemia and B-16 melanocarcinoma, respectively (2), may be attributed to preferential hydrolysis to the corresponding phenol (II) (R_1 = 3-OH; R_2 = H) in the tumors. The inactivity of the precursor phenol (II) (R_1 = 3-OH; R_2 = H) may have been due to facile detoxification *via* the hydroxyl group prior to reaching the tumor. The synthesis of the corresponding *O*-benzyl ethers (V) was planned, since in simple nonbiological systems, many phenyl alkyl ethers regenerate the corresponding phenol under acidic conditions (8) but are stable under mildly alkaline conditions. In addition, alteration of the Hammett values of the nuclear substituents of the benzyl group in series V may permit variation in the rate of release of the precursor phenol (9) which may correlate with differences in antineoplastic activity. In the case of VI, the loss of the benzyl groups *in vivo* would permit the formation of an aromatic compound with vicinal hydroxy groups which may be con-

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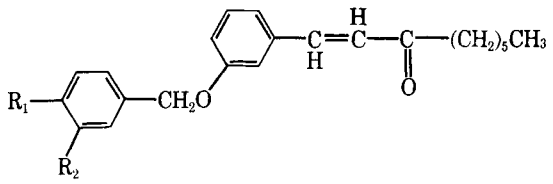
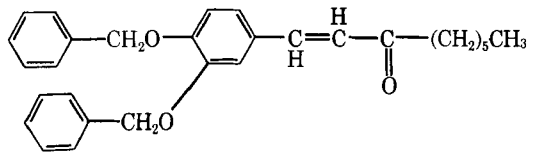
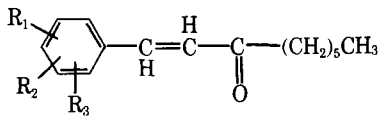


V

a: $R_1 = R_2 = H$
b: $R_1 = H$, $R_2 = Cl$
c: $R_1 = R_2 = Cl$
d: $R_1 = Cl$; $R_2 = H$
e: $R_1 = NO_2$; $R_2 = H$
f: $R_1 = CH_3$; $R_2 = H$



VI



VII

a: $R_1 = 2\text{-}OCH_3$; $R_2 = R_3 = H$
b: $R_1 = 4\text{-}OCH_3$; $R_2 = R_3 = H$
c: $R_1 = 2\text{-}OCH_3$; $R_2 = 3\text{-}OCH_3$; $R_3 = H$
d: $R_1 = 2\text{-}OCH_3$; $R_2 = 4\text{-}OCH_3$; $R_3 = H$
e: $R_1 = 3\text{-}OCH_3$; $R_2 = 5\text{-}OCH_3$; $R_3 = H$
f: $R_1 = 2\text{-}OCH_3$; $R_2 = 3\text{-}OCH_3$; $R_3 = 4\text{-}OCH_3$
g: $R_1 = 2\text{-}OCH_3$; $R_2 = 4\text{-}OCH_3$; $R_3 = 5\text{-}OCH_3$
h: $R_1 = 2\text{-}OCH_3$; $R_2 = 4\text{-}OCH_3$; $R_3 = 6\text{-}OCH_3$
i: $R_1 = 3\text{-}OCH_3$; $R_2 = 4\text{-}OCH_3$; $R_3 = 5\text{-}OCH_3$

verted to an *ortho*-quinone. Since the two dimethoxy compounds (III*b* and *c*) were shown to possess some activity against P-388 lymphocytic leukemia, the question as to their mode of action was raised. *O*-Demethylation of phenols is a common metabolic pathway (10), and thus, the formation of the corresponding dihydroxy compounds could occur with subsequent oxidation to the corresponding *para*- and *ortho*-quinones (Scheme I), which may then interact with important cellular nucleophiles such as thiols (11, 12). Thus, the preparation of further nuclear methoxy styryl ketones (VII) was suggested, and in the absence of nuclear hydroxylation by metabolism, compounds VII*c*, *f*, *g*, and *i* are able to be converted *in vivo* into quinones, in contrast to VII*d*, *e*, and *h*.

In addition, while 1-phenyl-1-nonen-3-one (II, $R_1 = R_2 = H$) and the related dichloro derivative (II, $R_1 = 3\text{-}Cl$; $R_2 = 4\text{-}Cl$) are inactive against P-388 lymphocytic leukemia, branching of the alkyl chain α to the carbonyl group led to compounds IV*a* and *b* with perceptible beneficial responses in this screen (3). The increase in bioactivity could be due to a number of factors: Alignment at a receptor site with these branched-chain compounds may be favored, and also differences in the hydrophilic–lipophilic properties of IV*a* and *b* compared with the compounds containing the *n*-hexyl group are conceivable. Thus, increased branching of the alkyl chain in the case of three isomeric *n*-pentanols led to increased aqueous solubility (13), and a recent generalization that the biological activities of α,β-unsaturated carbonyl compounds depends largely on stereochemical considerations and modifications of the hydrophobic portion of the molecules (14) is of relevance. Furthermore, by altering the branching of the four-carbon alkyl group in compounds VIII*a–c* and *e–g*, the degree of enolization would be predicted to vary (15, 16), which in turn would affect the hydrophilic–lipophilic balance. In addition, since the bioactivities of α,β-unsaturated ketones are considered to be due to reaction in part with cellular nucleophiles (17, 18), chemical reaction would be expected to be reduced with increasing enolization. The cyclohexyl compounds (VIII*d* and *h*), like VIII*b* and *f*, have secondary carbon atoms adjacent to the carbonyl group, but differences in hydrophilic–lipophilic properties would be expected [*e.g.*, the aqueous solubilities of cyclohexanol and *s*-butanol at 20° are 3.6 and 12.5%, respectively (19)].

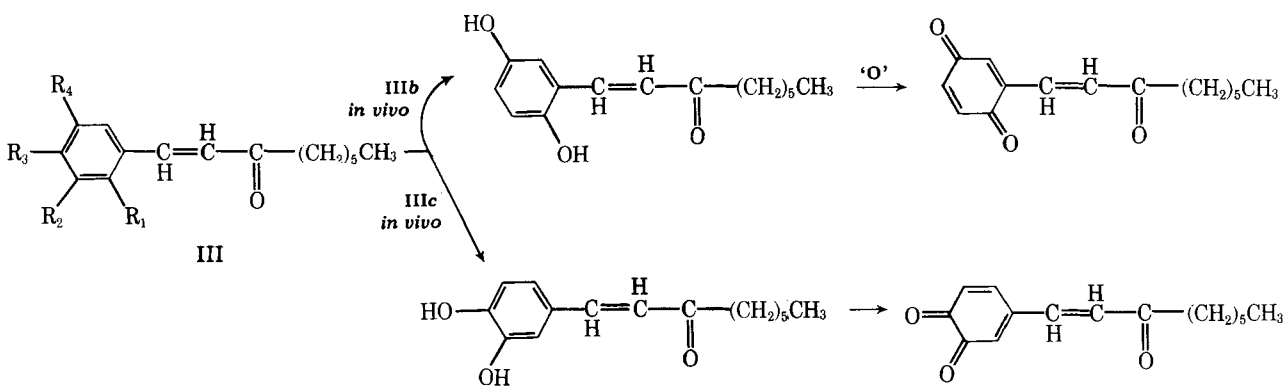
It has been shown recently that unsaturated alkyl ester groups, two carbon atoms distant from the carbonylene group, gave rise to compounds with antitumor properties, including activity against P-388 lymphocytic leukemia (20, 21), and hence, evaluation of the biological activity of series IX was deemed profitable.

Finally, the synthesis of prodrugs of 4-phenyl-but-3-en-2-one (IV*c*) was contemplated since the compound increased the median survival time in mice with P-388 lymphocytic leukemia by 26% (22). Since oximes may be prodrugs of ketones (23) and are known to be acid-labile (24), compound X may breakdown preferentially to IV*c* under the acidic conditions of certain neoplastic tissue. In addition, the dienol ether and related thio analog (XI*a* and *b*) would be expected to be stable under alkaline conditions but to be extremely sensitive to acid (25). The synthesis of these ethers (XI), if successful, could be applied to various Mannich bases (I) as well.

The second phase of the current investigation was to examine the metabolic route of III*a*. Primarily, this was to compare the antineoplastic activities of the metabolite(s) with the parent compound, and secondly, to observe the effect on anticancer properties of molecular modification of III*a* designed to retard metabolic processes.

The synthesis of compounds V–IX was achieved, and the effect on P-388 lymphocytic leukemia in mice for virtually all of the compounds is recorded in Table I. The *O*-benzyl ethers (V and VI), although not causing fatalities to mice under the test conditions, were inactive. An assessment of the stability of three of the ethers, (V*a*, *e*, and *f*) showed them to be stable in acetonitrile–phosphate buffer at physiological pH as well as at pH values of 6.9 and 6.4, which are likely to be found in various tumors. It is conceivable that a breakdown of the ethers V and VI to the corresponding phenols does not occur *in vivo*. None of the remaining ethers (VII) or ketones (VIII and IX) met the criterion for activity in the P-388 screen. Under the test conditions, no fatalities were noted at the maximum dose levels administered (200 mg/kg) for the compounds in Table I with the exception of VIII*g* and *h*. Oximation of the ketone IV*c* led to X which was nontoxic and inactive.

Attempts to synthesize the enol ether (XI*a*) were unsuccessful. Initially, a literature method used in the preparation of steroidal enol ethers (30) was employed but only unreacted ketone was isolated from the reaction mixture. When (*E*)-4-phenyl-3-buten-2-one and dimethoxypropane were heated in the presence of zinc chloride, two yellow oils, in ad-



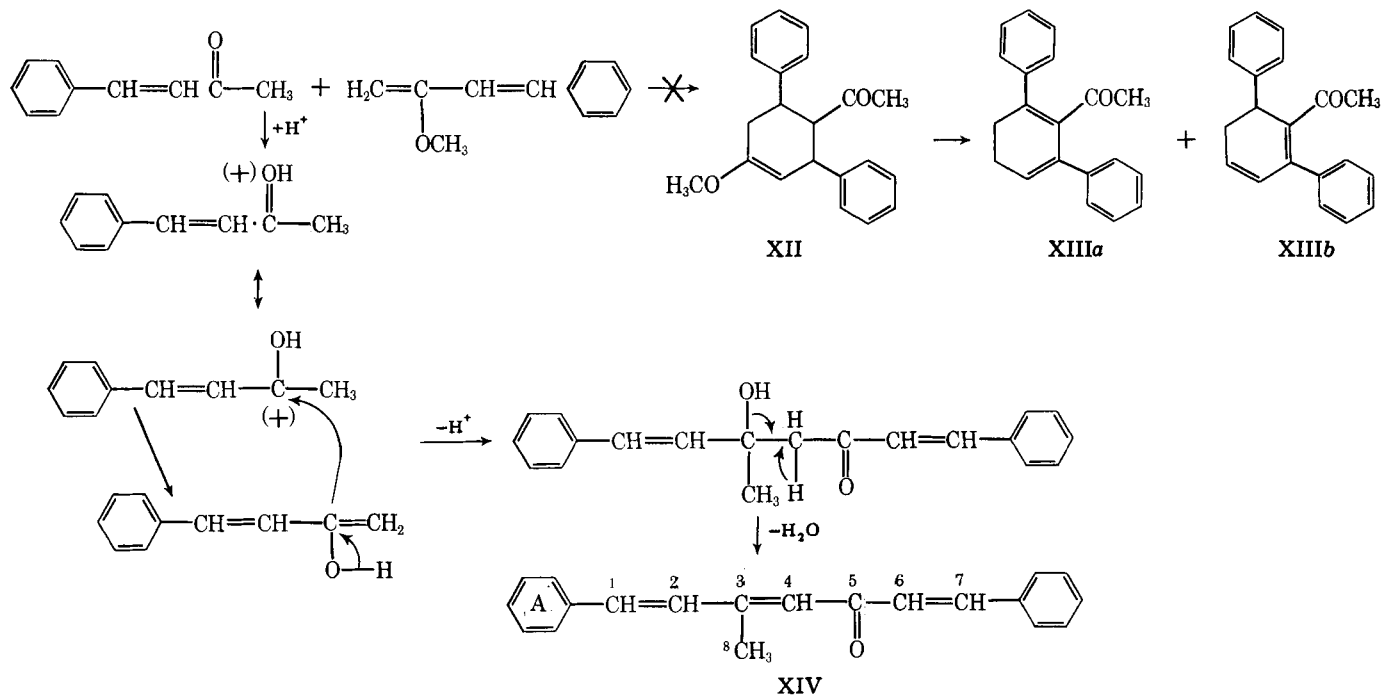
Scheme I

Table I—Physical Data on the Conjugated Styryl Ketones V–IX and Activity Against P-388 Lymphocytic Leukemia in Mice

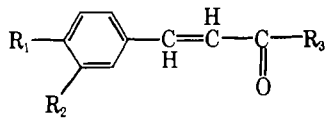
Compound	Yield, %	Melting Point or Boiling Point, °C/mm	Formula	Analysis Calc. C	Calc. H	Found C	Found H	Max Increase in Median Survival Time[a] (Dose in mg/kg)
V*a*	35	55	$C_{22}H_{26}O_2$	81.99	8.07	82.07	8.19	NA
V*b*	49	27–28	$C_{22}H_{25}ClO_2$	74.16	7.02	74.12	7.01	96(200)
V*c*	30	57	$C_{22}H_{24}Cl_2O_2$	67.52	6.18	67.80	6.05	115(50)
V*d*	46	46	$C_{22}H_{25}ClO_2$	74.16	7.02	74.03	7.04	101(200)
V*e*	54	74–75	$C_{22}H_{25}NO_4$[b]	71.91	6.86	71.60	6.81	91(50)
V*f*	62	42	$C_{23}H_{28}O_2$	82.03	8.32	82.15	8.55	NA
VI	54	78–79	$C_{29}H_{32}O_3$	81.32	7.47	71.81	7.12	92(12.5)
VII*a*	53	145–146/0.45	$C_{16}H_{22}O_2$	78.00	9.01	77.93	9.04	117(200)
VII*b*	21	54	$C_{16}H_{22}O_2$	78.00	9.01	78.17	9.08	103(100)
VII*c*	39	146–148/0.24	$C_{17}H_{24}O_3$	73.88	8.75	74.12	8.74	107(100)
VII*d*	48	71	$C_{17}H_{24}O_3$	73.88	8.75	74.06	8.54	94(50)
VII*e*	56	66	$C_{17}H_{24}O_3$	73.88	8.75	73.64	8.39	103(100)
VII*f*	31	32	$C_{18}H_{26}O_4$	70.56	8.55	70.47	8.58	102(200)
VII*g*	72	90	$C_{18}H_{26}O_4$	70.56	8.55	70.66	8.59	100(50)
VII*h*	65	110	$C_{18}H_{26}O_4$	70.56	8.55	71.09	8.54	101(100)
VII*i*	44	80	$C_{18}H_{26}O_4$	70.56	8.55	70.68	8.48	101(50)
VIII*a*	66	37[c]	$C_{13}H_{16}O$	82.93	8.57	82.87	8.58	108(200)
VIII*b*	58	89–91/0.37	$C_{13}H_{16}O$	82.93	8.57	82.88	8.49	114(200)
VIII*c*	31	37[d]	$C_{13}H_{16}O$	82.93	8.57	83.08	8.77	105(200)
VIII*d*	51	57	$C_{15}H_{17}O$	84.46	8.04	84.49	8.32	107(100)
VIII*e*	49	49[e]	$C_{13}H_{14}Cl_2O$	60.72	5.49	60.72	5.52	98(50)
VIII*f*	54	34–35	$C_{13}H_{14}Cl_2O$	60.72	5.49	60.77	5.45	99(200)
VIII*g*	34	89[f]	$C_{13}H_{14}Cl_2O$	60.72	5.49	60.72	5.57	107(100)[g]
VIII*h*	45	65	$C_{15}H_{15}Cl_2O$	63.84	5.34	63.68	5.77	103(50)[g]
IX*a*	81	Oil	$C_{18}H_{22}O_3$	75.49	7.75	75.84	7.80	107(100)
IX*b*	83	Oil	$C_{19}H_{24}O_3$	75.97	8.05	75.87	7.80	100(200)
IX*c*	85	Oil	$C_{19}H_{24}O_3$	75.97	8.05	76.17	8.02	98(50)
IX*d*	95	Oil	$C_{24}H_{26}O_3$	79.53	7.23	79.80	7.19	96(50)

[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 median survival time by 20% to be considered active. NA result is not yet available. [b] *Anal.*—Calc. for $C_{22}H_{25}NO_4$: N, 3.81. Found: N, 3.78. [c] Lit. (26) mp 38–39°. [d] Lit. (27) mp 41°. [e] Lit. (28) mp 49°. [f] Lit. (29) mp 89–91°. [g] There were 5/6 and 6/6 survivors on Day 5 at dose levels of 200 and 100 mg/kg, respectively.

dition to unreacted ketone, were obtained. Separation of the three components showed that the molecular ions of both yellow oils was 274, and both purified compounds reverted to an equilibrium mixture of these two compounds. Initially, it was considered that a Diels-Alder reaction between the desired product (XI*a*) and unreacted ketone (IV*c*) had occurred to give a cyclohexene derivative such as XII (or alternatively, where the aromatic rings are vicinal to each other), which subsequently lost a molecule of methanol to give cyclohexadienes, which may be represented by structures XIII*a* and *b* (Scheme II). Interconversion between XIII*a* and *b* is possible since similar molecular rearrangements in conjugated systems are known (31). However, an alternative explanation was considered in that under the reaction conditions which contain a Lewis acid, two molecules of protonated ketone could undergo an aldol condensation, which on dehydration would lead to the triolefine (XIV) also possessing a molecular weight of 274 (Scheme II). This compound could undergo (*E*)–(*Z*) isomerization at the double bonds in the molecule. Recourse to ^{13}C-NMR spectroscopy supported the structures of the yellow compounds as XIV, based on a comparison of the observed values of carbon absorptions with the calculated values of chemical shifts calculated from tables (32). The presence of XII was eliminated since no

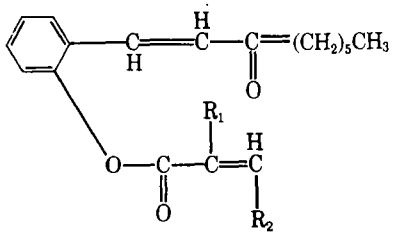


Scheme II



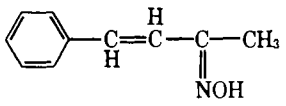
VIII

a: $R_1 = R_2 = H$; $R_3 = (CH_2)_3CH_3$
b: $R_1 = R_2 = H$; $R_3 = CH(CH_3)C_2H_5$
c: $R_1 = R_2 = H$; $R_3 = C(CH_3)_3$
d: $R_1 = R_2 = H$; $R_3 = C_6H_5$
e: $R_1 = R_2 = Cl$; $R_3 = (CH_2)_3CH_3$
f: $R_1 = R_2 = Cl$; $R_3 = CH(CH_3)C_2H_5$
g: $R_1 = R_2 = Cl$; $R_3 = C(CH_3)_3$
h: $R_1 = R_2 = Cl$; $R_3 = C_6H_5$

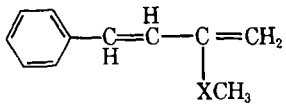


IX

a: $R_1 = R_2 = H$
b: $R_1 = H$; $R_2 = CH_3$
c: $R_1 = CH_3$; $R_2 = H$
d: $R_1 = H$; $R_2 = C_6H_5$

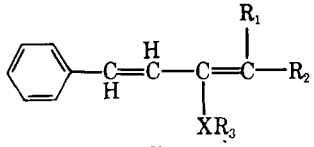


X



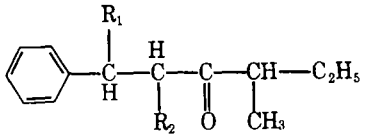
XI

c: X = O
b: X = S



XV

a: $R_1 = H$, $R_2 = (CH_2)_4CH_3$; $R_3 = CH_3$; X = O
b: $R_1 = R_2 = H$; $R_3 = C_2H_5$; X = S
c: $R_1 = CH_3$; $R_2 = C_2H_5$; $R_3 = C_6H_5$, X = S
d: $R_1 = R_2 = H$; $R_3 = C_2H_5$; X = O



XVI

a: $R_1 = SC_6H_5$; $R_2 = H$
b: $R_1 = R_2 = Br$

absorbances at 30–40 ppm or 200–207 ppm were observed, indicating the absence of methylene and acetyl carbon atoms, respectively. No reaction was observed when II ($R_1 = R_2 = H$) was reacted with dimethoxypropane under the same experimental conditions in an attempt to produce XV*a*.

In an attempt to produce the thio ether (XV*b*) using a modification of the literature procedure (30), principally unreacted ketone (IV*c*) was found as well as two unidentified components. It has been observed that formation of thioenol ethers, in contrast to mercaptans, is enhanced by the carbon atom α to the carbonyl group being sterically hindered and having an electron-releasing group attached to it (33). These workers used thiophenol rather than ethanethiol, and hence, the reaction between VIII*b* and thiophenol was attempted. Examination of the product showed the presence of principally unreacted ketone (VIII*b*), the Michael adduct, 4-methyl-1-phenyl-1-phenylthiohexan-3-one (XVI*a*), and an unidentified product. To eliminate the formation of this Michael adduct, the dibromoketone (XVI*b*) was synthesized, but no reaction between this compound and thiophenol occurred under the experimental conditions employed.

Finally, a related enol ether (XV*d*) was synthesized essentially by literature procedures (34, 35) and shown to revert rapidly to 4-phenyl-3-buten-2-one (IV*c*). The marked instability of this compound precluded its assessment against P-388 lymphocytic leukemia. Hence, even if the desired compounds (XI) were prepared, it may be that they would be too unstable for pharmacological evaluation, even if the idea of XI reverting to 4-phenyl-3-buten-2-one is validated.

In an attempt to study the metabolism of III*a*, this compound was administered intraperitoneally into rats. No trace of III*a* nor metabolites was found in the urine and feces. Examination of rat plasma at different time intervals after intraperitoneal injection of III*a* did not reveal the presence of III*a* or metabolites, and it was considered that they may have been bound extensively to proteins [*e.g.*, an aromatic hydroxy compound used as a cholecystographic medium has been shown to be bound by covalent bonds to plasma protein albumin with a half-life of 2.5 years (36)]. However, analysis of plasma revealed the absence of xenobiotics. Since gross physiological effects were observed in the animals soon after intraperitoneal injection of III*a*, it was felt that this compound was being absorbed rapidly, so III*a* was administered by the intravenous route and blood obtained after short time intervals. Five minutes after injection of III*a*, the compound was detected, but after 10 min the concentration of this compound had been reduced 400 times compared to the quantity of III*a* measured at the end of 5 min. Since neither III*a* nor metabolites were found in the urine or feces, it was thought that rapid sequestration to adipose tissue in part could occur due to the lipophilicity of III*a*. Extraction of rat brain 15 min after intraperitoneal injection revealed the presence of ~5% of III*a*. This observation demonstrates that III*a* crosses the blood–brain barrier and is present in brain tissue not in the covalently bound state. This passage through the blood–brain barrier by III*a* may permit it to serve as a prototype of a series of compounds for evaluation against brain tumors. It is conceivable that this compound primarily is distributed to and retained by the fatty tissue of the rat.

After 6 weeks, some of the animals that had been dosed with III*a* died, and the remaining animals appeared sickly. The live animals were euthanized, and the gross pathological examination revealed peritonitis as well as enlarged livers and spleens. In addition, blood was found in various body cavities, and, although it did not clot on exposure to air, the prothrombin and partial thromboplastin times as well as platelet counts were normal. The capillaries in the reticular surface collagen surrounding livers and spleens seemed to be inadequately supported and may have permitted blood seepage into the organ capsules and abdomen. Changes in the mesenteric lymph nodes and the fact that no evident source of hemorrhage was found suggest that the bleeding into the abdomen noted in this group of treated rats occurred over several days. Histological examination revealed significant abnormalities in the liver and spleen. Figures 1 and 2 show the hepatic necrosis observed and the damage to the splenic capsules, respectively. In order to evaluate whether the pathological changes occurred soon after injection or gradually over a period of time, rats were injected with III*a* and euthanized at the end of 1, 2, 3, and 4 weeks. In general, the pathological changes noted were progressive. Finally, the dose of III*a* was reduced tenfold, and examination at the end of 4 weeks revealed cellular damage in the liver and spleen.

EXPERIMENTAL

Melting points and boiling points are uncorrected. Elemental analyses were undertaken locally[1], and the aluminum oxide[2] and silica gel[3] used in column chromatography were obtained commercially. TLC was carried out using aluminum oxide and silica gel with fluorescent indicator sheets[4],

[1] R. E. Teed, Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.
[2] Alcoa Chemicals aluminum oxide (F-20), Aluminum Co. of Canada.
[3] Silica gel 28-200 mesh, Sargent-Welch Scientific Co.
[4] Eastman Kodak Co.

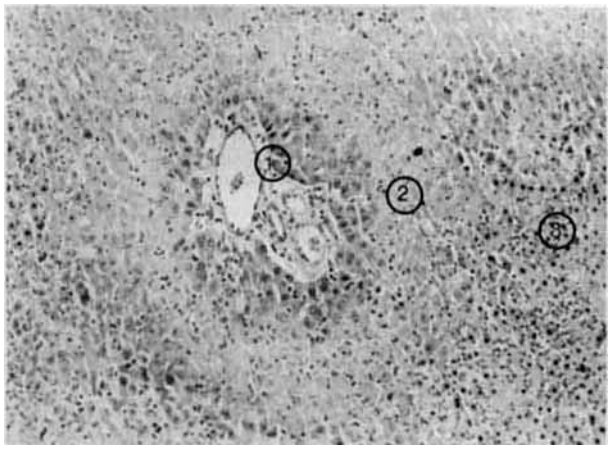


Figure 1—*Rat liver showing a viable portal triad ①, surrounded by an area of coagulation necrosis ②, and inflammation ③ (140×).*

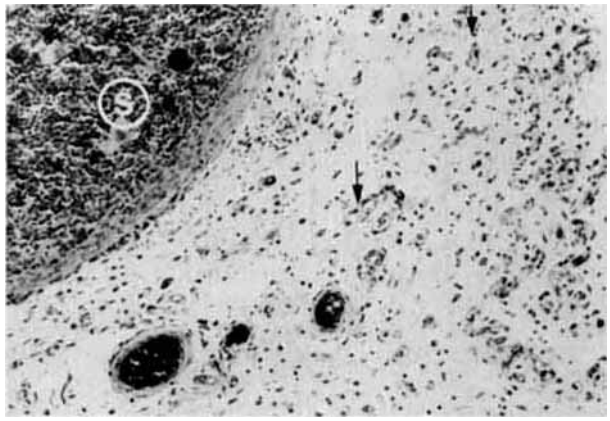


Figure 2—*Rat spleen (S) showing a thickened capsule composed of very fine reticular connective tissue and many poorly supported capillaries (arrows) (220×).*

while silica gel[5] (0.5-mm thick) on glass was used in preparative TLC. The plates were conditioned at 120° overnight and cooled prior to use. Unless otherwise stated, the boiling point of petroleum ether was 40–60°. Mass spectra[6-8] were run at 70 eV and the 60 MHz[9,10] and ^{13}C-NMR spectra[10] were determined using tetramethylsilane as the internal standard. GLC[11,12] analysis utilized a 122-cm × 3-mm column packed with phenylmethylsilicones on film calcined diatomite material of low density[13]. The injection port and detector temperatures were 250 and 300°, respectively, and the chromatograms were programmed from 150 to 250° at 4°/min. The physical data for Compounds V–IX are found in Table I.

1-(*m*-Benzyloxyphenyl)-1-nonen-3-ones (V*a–f*)—The compounds were prepared by alkylation of 1-(*m*-hydroxyphenyl)-1-nonen-3-one (2) with the appropriate benzyl chloride using a literature procedure (37) to give the crude product as a brown oil, purified as follows: The unsubstituted compound (V*a*) was chromatographed using a column of alumina and eluted with petroleum ether, followed by benzene and finally methanol. Recrystallization from petroleum ether afforded V*a* as a colorless powdery material. Compound V*b* was purified by trituration with cold petroleum ether to give colorless crystals which were recrystallized from petroleum ether. Compounds V*c* and *d* were purified by chromatography using alumina and a solvent system of chloroform–ethyl acetate–diethylamine (92:5:3). After the products were eluted, they were placed in a freezer (−5°) overnight and the solids obtained recrystallized from petroleum ether to give V*c* and *d* as a colorless powdery material. The nitro analog (V*e*) was purified by placing the crude oil in the freezer overnight and the crystals formed were recrystallized from anhydrous diethyl ether to give V*e* as a colorless product. The crude oil containing V*f* was placed in the freezer overnight and the crystals formed triturated with cold methanol. Recrystallization from methanol afforded V*f* as a colorless powdery material.

The stabilities of V*a*, *e*, and *f* in acetonitrile–phosphate buffers (1:1), which were prepared using the described methodology (38), were demonstrated as follows: The ketone (10^{-3} *M*) was dissolved in the buffer (250 ml) at pH values of 7.4, 6.9, and 6.4. The spectrum of the compound was obtained at dissolution ($t = 0$) and the solution retained at 37 ± 0.1° for 24 hr and monitored at regular time intervals. No change in the absorption at λ_{max} was noted.

1-(3,4-Dibenzyloxyphenyl)-1-nonen-3-one (VI)—A solution of 3,4-dibenzyloxybenzaldehyde (3.0 g, 0.010 mole), 2-octanone (1.4 g, 0.011 mole), piperidine (0.86 g, 0.01 mole), and glacial acetic acid (0.66 g, 0.011 mole) in benzene (50 ml) was heated under reflux for 36 hr. A Dean-Stark trap was attached to the reaction vessel. On cooling the solution was washed with water (3 × 20 ml) and the organic phase dried (anhydrous magnesium sulfate). After evaporation of benzene, the residue was cooled over ice to produce yellow crystals (2.6 g). Purification was accomplished by column chromatography using silica and benzene–acetone (4:1) to give VI as colorless needles (2.3 g). TLC using silica and developing with methylene chloride revealed one spot, $R_f = 0.80$. The mass spectrum of VI showed *m/z* 428 (M^+ 8%) and 91 (100%). NMR ($CDCl_3$): δ 7.30 (m, 13, aromatic H), 7.10 (d, 1, C_1H, J = 16 Hz), 6.55 (d, 1, C_2H, J = 16 Hz), 5.12 (s, 4, 2 × $CH_2C_6H_5$), 2.60 (t, 2, C_4H), and 1.40 [m, 11, $(CH_2)_4CH_3$]. Repeated elemental analyses invariably gave similar results to those quoted in Table I. Only unreacted aldehyde was isolated in an attempt to synthesize VI by the method employed for the preparation of V.

Methoxy-Substituted 1-Phenyl-1-nonen-3-ones (VII)—These ketones were prepared by a literature method (39). The yellow and colorless compounds obtained were recrystallized from methanol, except for VII*d* and *e*, which were recrystallized from petroleum ether.

Styryl Alkyl Ketones (VIII)—These compounds were prepared by the literature procedure (39). The crude products were recrystallized from petroleum ether to yield colorless crystals, except VIII*b*, which was distilled to give the desired compound as a pale yellow oil.

Esters of 1-(*o*-Hydroxyphenyl)-1-nonen-3-one (IX)—The esters (IX) were prepared from 1-(*o*-hydroxyphenyl)-1-nonen-3-one (40) and the appropriate acid chloride in the presence of triethylamine using a literature method (41), except that after the reactants were stirred at room temperature, diethyl ether was added to precipitate all of the triethylamine hydrochloride. Evaporation of the solvent produced yellow oils, which were passed through a column of alumina using methylene dichloride as the eluting solvent to give IX*a* and c as yellow oils, IX*b* as a pink oil, and IX*d* as a pale brown oil which solidified on standing. TLC using alumina and petroleum ether (bp 30–60°) as the eluting solvent showed only one spot.

(*E*)-4-Phenyl-3-buten-2-one oxime (X)—A mixture of (*E*)-4-phenyl-3-buten-2-one (5.0 g, 0.034 mole), hydroxylamine hydrochloride (1.2 g, 0.017 mole), sodium acetate (3.1 g, 0.038 mole), ethanol (25 ml), and water (25 ml) was heated under reflux for 5 hr. Water (200 ml) was added, and after cooling, the mixture was extracted with ether (3 × 50 ml) and the organic extracts washed with water, dried (anhydrous magnesium sulfate), and removal of the solvent afforded a yellow oil which solidified on standing. Recrystallization from petroleum ether (bp 40–60°) gave X as fawn crystals, mp 109° [lit. (42) mp 110°]; 52% yield.

Attempted Synthesis of (*E*)-3-Methoxy-1-phenyl-1,3-butadiene (XI*a*)—Conversion of (*E*)-4-phenyl-3-buten-2-one (IV*c*) into XI*a* was attempted initially using a literature procedure (30), but only unreacted ketone was isolated from the reaction mixture. The result was identical when the time of heating under reflux was extended from 3.5 to 24 hr. Employment of a published method (43) substituting dimethoxypropane for benzylmercaptan, gave a yellow-brown oil, which was shown by TLC using silica and a developing solvent of methylene chloride–benzene (4:1) to consist of unreacted ketone (IV*c*) $R_f = 0.44$ and two yellow components, $R_f = 0.52$ and 0.61. After replacement of the zinc chloride by stannic chloride, the reaction was monitored for 72 hr by TLC to observe the ratio of unreacted IV*c* to the two yellow compounds. After 3.5 hr, this ratio appeared to be constant. Attempts to separate the two yellow components by column chromatography using silica and a mixture of

[5] Silica gel GF Woelm TLC, ICN Pharmaceuticals, GMbH and Co., West Germany.
[6] AE1 MS-12 mass spectrometer, Picker X-Ray Engineering Ltd.
[7] VG Micromass MM16F mass spectrometer with 2025 data system.
[8] Finnigan model 4000 gas chromatograph mass spectrometer interfaced to a Finnigan Incos model 2300 data system. Samples were injected splitless on a 50 m × 0.3-mm fused silica capillary column coated with OV-1 at room temperature and the temperature raised ballistically to 150° and then programmed at 4°/min to 275°. The mass spectrometer was scanned from mass 40 to mass 650 every 2 sec. The temperature of the injector and separator oven were both at 250°.
[9] Varian T-60 spectrophotometer, Varian Associates of Canada Ltd.
[10] Brucker WP-60 spectrophotometer, Brucker Spectrospin (Canada) Ltd.
[11] Hewlett-Packard 5750 gas chromatograph.
[12] Perkin-Elmer Sigma 3B gas chromatograph.
[13] 3% OV-17 on Chromosorb W.

methylene chloride and benzene (4:1) were unsuccessful. Preparative TLC using silica (0.5 mm) and a developing solvent of methylene chloride and benzene (4:1) gave two distinct yellow bands which were eluted with methylene dichloride and the solvent removed *in vacuo* at room temperatures. Evaporation of the solvent gave the two compounds and were subject to mass spectral and ^{13}C-NMR evaluations. After refrigeration with protection from light for 24 hr, both compounds had reverted to an identical mixture of two yellow derivatives. The molecular ions of both compounds were 274 (100%). The ^{13}C-NMR chemical shifts were determined in deuterochloroform and are consistent with structure XIV (assignment, calculated value): 136.61 (C-1 aromatic ring A, 137.3); 128.18 (C-2 aromatic ring A, 127.9); 130.09 (C-3 aromatic ring A, 130.5); 126.39 (C-4 aromatic ring A, 126.2); 129.47 (C-1, 129.8); 135.13 (C-2, 135.3); 151.63 (C-3, 153.9); 125.35 (C-4, 123.9); 190.17 (C-5, –); 142.03 (C-6, 144.5); 150.34 (C-7, 151.1); and 14.30 (C-8, –) ppm. A further attempt to produce XI*a* by a literature procedure (44) produced an orange-brown oil shown by TLC [silica and a developing solvent of methylene chloride and benzene (4:1) to consist of unreacted ketone, R_f = 0.44, and two yellow components, R_f = 0.52 and 0.61].

Attempted Syntheses of the Butadienes (XV*a–c*)—An attempted conversion of (*E*)-1-phenyl-1-nonen-3-one (39) into the corresponding butadiene (XV*a*) by the reported procedure (43) led to the isolation of unreacted ketone from the reaction mixture (TLC evidence using silica and methylene chloride as the developing solvent). In the attempted synthesis of XV*b* from (*E*)-4-phenyl-3-buten-2-one (IV*c*) and ethanethiol by the published procedure (43), only unreacted ketone was isolated from the reaction mixture as revealed by TLC (silica and methylene chloride). Modification of a literature procedure (30) in a further attempt to produce XV*b* was as follows: A mixture of IV*c* (5.72 g, 0.035 mole), ethanethiol (2.67 g, 0.043 mole), and *p*-toluenesulfonic acid (0.50 g, 0.003 mole) in benzene (100 ml) were heated under reflux for 24 hr. On cooling, the mixture was neutralized with sodium bicarbonate (1.5 g), added to ice water (200 ml), and stirred at room temperature for 0.5 hr. After extraction with benzene, the organic layer was washed with water, dried (anhydrous sodium sulfate), and removal of benzene afforded a brown oil (3.6 g). TLC on silica using methylene chloride as the developing solvent revealed the presence of unreacted ketone, R_f = 0.55, and two yellow components, R_f = 0.69 and 0.80.

The attempted synthesis of the thioether (XV*c*) from 4-methyl-1-phenyl-1-hexen-3-one, VIII*b*, and thiophenol using the published procedure (33) yielded a yellow oil. TLC of the product using silica and a developing solvent of cyclohexanone–benzene (94:6) revealed the presence of unreacted ketone, R_f = 0.60, and two other compounds, R_f = 0.72 and 0.83. Separation of the components by preparative TLC using silica and cyclohexanone–benzene (94:6) was achieved and the compound with an R_f value of 0.72 was identified as 4-methyl-1-phenyl-1-phenylthiohexan-3-one (XVI*a*) as a yellow oil, NMR ($CDCl_3$): δ 7.05 (m, 10, aromatic H); 4.75 (t, 1, C_1H, J = 7 Hz); 3.00 (d, 2, C_2H, J = 7 Hz); 2.3 (broad q, 1, C_4H, J = 7 Hz); 1.40 (m, 2, C_5H); and 0.80 (m, 6, C_4HCH_3, C_6H).

Anal.—Calc. for $C_{19}H_{22}OS$: C, 76.46; H, 7.43. Found: C, 76.41; H, 7.49.

The remaining compound, R_f = 0.83, was not identified. However, mass spectrometry revealed the molecular weight to be 280 and the NMR spectrum showed the presence of aromatic protons and various aliphatic hydrogen atoms.

In an attempt to prepare the dibromo analog of XV*c*, the required intermediate ketone, 1,2-dibromo-4-methyl-1-phenyl-3-hexanone (XVI*b*), was prepared by a literature method (45) to give the desired product as colorless needles (mp 97.5°, 64% yield).

Anal.—Calc. for $C_{13}H_{16}Br_2O$: C, 44.82; H, 4.60. Found: C, 44.79; H, 4.60.

Reaction of 1,2-dibromo-4-methyl-1-phenyl-3-hexanone with thiophenol by the literature method (33) was unsuccessful and only unreacted ketone was isolated from the reaction mixture, mp 96–97°.

3-Ethoxy-1-phenyl-1,3-butadiene (XV*d*)—*n*-Butyl lithium (0.14 g, 0.002 mole) in hexane (0.64 ml) was added dropwise to a vigorously stirred suspension of 2-ethoxypropenyl-triphenylphosphine iodide (34) (0.948 g, 0.002 mole) in anhydrous tetrahydrofuran (10 ml) under nitrogen which was cooled to −50°. After the addition was complete, the orange mixture was allowed to warm to −25° and stirring was continued for 2 hr. On cooling to −78°, benzaldehyde (0.212 g, 0.002 mole) was added and the mixture stirred at this temperature for 15 min, 0° for 2 hr, and overnight at room temperature. After heating the reaction mixture under reflux for 6 hr, it was diluted with water (40 ml) and extracted with hexane. The hexane extracts were combined, washed with water, and dried (anhydrous magnesium sulfate), and evaporation of the organic solvent afforded XV*d* as a brown oil (0.15 g). NMR ($CDCl_3$) spectrum was identical to the published data for the (*E*) and (*Z*) isomers of XV*d* (35, 46). On standing overnight at room temperature in a sealed dark container, IV*d* was shown by mass spectral, NMR, and TLC evidence to have been converted into (*E*)-4-phenyl-3-buten-2-one (IV*c*). Attempts at purification of the butadiene (XV*d*) by preparative TLC using silica and a hexane–ether mixture (60:40) caused a breakdown of XV*d* into IV*c*. Column chromatography on alumina and eluting with a hexane–ether mixture (60:40) did not permit purification of the components, and vacuum distillation caused the reaction product to decompose.

Screening of Compounds—The anticancer screening was carried out[14] using protocols described previously (47). The compounds were administered by the intraperitoneal route into either male or female CD_2F_1 mice and male $B_6D_2F_1$ mice. The compounds were administered for 9 consecutive days except in the case of V*b*, VI, IX*a–d*, and X, which were injected for 5 consecutive days. The compounds were administered as suspensions in saline with polysorbate 80[15] except for VIII*d*, *e*, *g*, and *h*, X, and XVI, which were injected in hydroxypropylcellulose and also Compounds IX*d* and V*b*, which were administered in saline with alcohol and in saline with polysorbate 80[15] plus alcohol, respectively.

Examination of the Effect of III*a* in Rats—Unless stated otherwise, III*a* was suspended in normal saline containing 3% polysorbate 80[15] and injected by the intraperitoneal route into male albino Wistar rats. Control animals were injected with normal saline containing 3% polysorbate 80[15].

Metabolism Studies—Dose of III*a* used in metabolism studies is as follows: It has been shown[16] that two doses of III*a* of 400 mg/kg given by the intraperitoneal route on Days 1 and 7 caused no mortalities in the $B_6D_2F_1$ strain of mice by Day 5, and initially it was decided to examine the effect of III*a* in rats at single dose levels of 400, 300, and 200 mg/kg over a 72-hr period. Injection of III*a* at the 400-mg/kg dose level into two rats caused an onset of action between 3 and 5 min. The animals remained inactive for 24 hr, laying on their sides. Only slight movement was initiated by physical stimuli and slight hemorrhaging was observed around the nose. During the first 24 hr, the urine output was 16% that of two control rats. After 48 hr the urine output was normal. Four rats dosed with 300 mg/kg of III*a* had a urine output similar to two control animals, although after 3–5 min, the animals appeared to be sedated. Slight hemorrhaging around the nose was noted after 24 hr, but after 48 hr the animals appeared normal. Two rats dosed with 200 mg/kg of III*a* displayed similar gross physiological effects as the animals dosed with 300 mg/kg of III*a*. Hence, a dose of 300 mg/kg was chosen for the metabolism studies.

Efficiency of Extraction of III*a* from Urine, Feces, Plasma, and Brain of Rats—The percent efficiency of extraction of III*a* from urine, feces, plasma, and brain of rats was determined by GLC by a modification of a literature procedure (48). Compound III*a* (0.04 mg) was added to control urine (2.00 ml), and after basification with aqueous sodium hydroxide solution (0.45 *N*) to pH 10.50, it was extracted with ether (2 × 5 ml). After evaporation of the organic solvent, the extract was dissolved in a solution (0.10 ml) of promazine hydrochloride in methanol (1.00 mg/ml). The peak height ratio of III*a*, promazine hydrochloride, recorded when 1 μl of this solution was injected onto a GLC column, was compared to the ratio obtained after injecting 1 μl of a solution containing 0.40 mg of III*a* and 1.00 mg of promazine hydrochloride/ml. It was observed that 98% of III*a* was recovered by this method.

Compound III*a* (8.00 mg) was added to 25 g of control feces and extracted by the literature method (48). The ether-soluble basic fraction was evaporated to dryness, dissolved in 100 μl of a solution containing II (R_1 = 3-OH; R_2 = H) in methanol (1.00 mg/ml), and the peak height ratio of III*a*, II (R_1 = 3-OH; R_2 = H), was compared with the peak height ratio of a 1-μl sample of a solution containing III*a* (8.00 mg) and II(R_1 = 3-OH; R_2 = H; 0.1 mg) in methanol/100 μl. The percentage of III*a* extracted by this method was 61%.

Compound III*a* (0.04 mg) was added to plasma (1 ml), basified with aqueous sodium hydroxide solution (0.45 *N*) to pH 11.5, and extracted with ether (2 × 5 ml). After the ether was evaporated, the extract was dissolved in 100 μl of a solution containing II (R_1 = 3-OH; R_2 = H) in methanol (1 mg/ml). The peak height ratios of III*a* with II (R_1 = 3-OH; R_2 = H) were compared with a 1-μl sample of a solution containing 0.04 mg of III*a* and 0.10 mg of II (R_1 = 3-OH; R_2 = H) in 100 μl of methanol. It was found that the efficiency of extraction of III*a* from plasma was 71%.

[14] Anticancer screening was carried out by the Drug Research and Development Division of the National Cancer Institute, Bethesda, Md.

[15] Tween-80, Atlas Chemical Laboratories.

[16] The National Cancer Institute, Bethesda, Md.

The brain of a rat was removed, homogenized[17], and after the addition of IIIa, the compound was extracted by the literature method (49), except that no basification was undertaken and the extraction solvent was ether, not 1-chlorobutane. Comparison of the peak heights of IIIa with II (R_1 = 3-OH; R_2 = H) by the method already described, indicated a percentage efficiency of extraction of IIIa of 75%.

Examination of Rat Urine and Feces for IIIa and Metabolites—Compound IIIa was injected into rats weighing between 200 and 300 g. Normally a trial consisted of 10 dosed rats and 5 control animals. The urine and feces were collected every 24 hr for 3 days and then at 1-week intervals for 4 subsequent weeks. If the samples were not utilized on the day of collection, they were frozen immediately at −5°. Both 24-hr test and control urine were extracted with ether and fractionated into the strongly acidic, weakly acidic, neutral, amphoteric, and basic components essentially by the literature procedure (48). The extracts were dissolved in methanol (100 μl) and a 1-μl sample injected onto a GLC column. No peaks were found in the extracts from the urine of animals dosed with IIIa, which also were not found in the extracts from the urine of the control animals. A similar observation was made after extracting the urine collected between 24 and 48 hr and also between 48 and 72 hr after injection of IIIa. Extraction of the 24-hr test and control feces (25 g) with ether was undertaken by the published procedure (48), the extracts were dissolved in methanol (100 μl), and a 1-μl sample injected onto a GLC column. The chromatograms generated did not reveal the presence of unchanged IIIa nor metabolites in the test feces. At this time, the toxicity of IIIa became apparent and the dose of IIIa was reduced tenfold. Samples of the 24, 48, and 72 hr and 1–4 week urine from six rats dosed with 30 mg/kg of IIIa and two control animals were extracted as described above, and no unchanged IIIa nor metabolites were found. Both test and control urine (2 ml) were adjusted to pH 5.2 with an acetate buffer and sulfatase[18] (20 μl containing β-glucuronidase) was added and the mixture was incubated at 37° overnight. Extraction and analysis by GLC indicated the absence of IIIa and related metabolites.

Examination of Rat Plasma after Intraperitoneal Injection of IIIa—Compound IIIa (30 mg/kg) was injected by the intraperitoneal route into rats weighing between 200 and 500 g. Rats were decapitated 15 min and 2, 24, and 48 hr after injection; the blood was collected and extracted by the following methods. First, test plasma (1.0 ml) was basified to pH 11.5 with aqueous sodium hydroxide solution (0.45 *N*), extracted with ether, and the organic extracts evaporated to dryness. The original plasma was then acidified with aqueous sulfuric acid (1 *N*) to pH 2.4, extracted with ether (2 × 5 ml), and the ether extracts evaporated to dryness (Method A). Second, plasma (1.0 ml) and 4 *N* aqueous HCl (1.0 ml) were incubated at 85° for 10 min. The plasma (pH 2.4) was extracted with ether (2 × 5 ml) and the ether removed *in vacuo*. The original plasma was then basified with aqueous sodium hydroxide solution (0.45 *N*) to pH 11.5, extracted with ether (2 × 5 ml), and the ether removed by evaporation. Third, plasma from dosed animals (1.0 ml) and 4 *N* aqueous HCl (1.0 ml) were incubated at room temperature for 24 hr. The plasma (pH 2.4) was extracted with ether (2 × 5 ml) and the ether evaporated. Basification of the original plasma to pH 11.5 was accomplished with aqueous sodium hydroxide solution (0.45 *N*), extracted with ether (2 × 5 ml), and the ethereal extract evaporated to dryness. Fourth, plasma (1.0 ml) was extracted by a literature procedure (36). In all cases control plasma was extracted by identical methods. Extracts from the plasma of dosed and control animals were dissolved in 100 μl of methanol and a 1-μl sample examined by GLC. Chromatograms of the dosed and control plasma extracts appeared identical.

Examination of Rat Plasma after Intravenous Injection of IIIa—Rats weighing between 200 and 250 g were injected into the femoral vein with IIIa (300 mg/kg). In the first experiment, blood was collected after 5 and 10 min, and in the second, blood was collected by cardiac puncture after 5 and 8 min. Plasma was separated and extracted by Method A. The extracts were dissolved in 100 μl of a solution of II (R_1 = 3-OH; R_2 = H) in methanol (1 mg/ml), and a 1-μl sample was analyzed by GLC. The quantity of IIIa in the plasma samples was determined by comparing the peak height ratio of IIIa to II (R_1 = 3-OH; R_2 = H) with a standard curve prepared from standard solutions of these two compounds. The results were determined by calibration and revealed that the concentrations of IIIa were 1.974 (2.132, 1.816), 0.602, and 0.005 mg/ml after 5, 8, and 10 min, respectively.

Examination of Rat Brain after Intraperitoneal Injection of IIIa—A dose of 300 mg/kg of IIIa was injected intraperitoneally into a rat weighing 340 g and the animal decapitated 15 min after injection. The brain was removed, homogenized[17], and the compound extracted by the literature procedure (49), except that basification prior to extraction was omitted, and the extraction solvent was ether, not 1-chlorobutane. The quantity of IIIa in the brain was measured using II (R_1 = 3-OH; R_2 = H) as the reference compound by the same procedure described earlier and found to represent 4.78% of the injected dose of the compound.

Pathological Study of IIIa—A dose of 300 mg/kg of IIIa was injected intraperitoneally into 10 male Wistar rats and two control animals were utilized. Deaths occurred in three animals after 40, 41, and 42 days, and at the end of 43 days the remaining seven animals were weak and bloated. There was paralysis in the hind legs and hemorrhaging from the nose. Necropsy was undertaken on these seven animals. Tissues were fixed in 10% buffered formaldehyde solution, sectioned at 5 μm, and stained with hematoxylin and eosin.

Blood samples were obtained by cardiac puncture prior to necropsy of the seven rats. Hemoperitoneum was noted in six of the seven rats. The markedly distended abdomens and scrotal sacs contained blood. Hemopericardium was noted in the seventh rat. The blood found in body cavities did not clot on exposure to air; however, the prothrombin times, partial thromboplastin times, and platelet counts of representative test animals were comparable to those of the controls. Two rats had a chronic diffuse peritonitis, whereas in the other five, the peritonitis was confined to the cranial abdomen where the diaphragm, liver, spleen, stomach, proximal duodenum, colon, and cranial poles of the kidneys adhered to one another by fibrous strands. Both the liver and spleen were enlarged in all of the rats examined. The stomach and intestines contained food and the other organs appeared normal.

The significant histological changes were found in the spleen, liver, and mesenteric lymph nodes. Chronic perihepatitis was characterized by a markedly thickened fibrous capsule. Loose granulation tissue attached to the peritoneal surface of the capsule contained capillaries, fibroblasts, and an infiltrate of polymorphonuclear leukocytes. Liver parenchymal changes were observed throughout all lobes and ranged from periportal vacuolation, basophilic cytoplasmic clumping, single hepatocyte necrosis, and occasional mitosis to multifocal centrilobular and midzonal coagulation necrosis. The periportal areas seemed to have been spared, although single necrotic cells were visible in these areas. Polymorphonuclear leukocytes were noted in the areas of necrosis.

Changes in the splenic capsule were similar to those in the liver capsule. The splenic red pulp was congested. Extramedullary hematopoiesis varied in degree among the rats from the occasional focus to more diffuse areas of hemopoietic activity.

Numerous erythrocytes were present in the medullary and subcapsular sinuses of the mesenteric lymph nodes. Siderocytes were numerous and erythrophagocytosis was evident.

To evaluate whether these pathological changes occurred soon after injection of the compound or gradually, four rats were injected intraperitoneally with IIIa at a dose of 300 mg/kg and euthanized for necropsy after 1, 2, 3, and 4 weeks. No gross abnormalities were noted in the rat 1 week postinjection. Mild hepatomegaly was noted 2 weeks postinjection. Perihepatitis, hepatomegaly, perisplenitis, and hemoperitoneum were noted 3 weeks postinjection, although the amount of blood in the abdominal cavity was very small. Perisplenitis, hepatomegaly, and congestion were noted 4 weeks postinjection. Liver parenchymal changes appeared to be progressive. Hydropic degeneration of periportal hepatocytes was mild 1 week postinjection but marked 2, 3, and 4 weeks postinjection. Over the 4 weeks, the vacuolation progressed towards the central veins. Mitotic figures were frequent in hepatocytes during the first 2 weeks postinjection but decreased during weeks 3 and 4. Many of the mitoses observed were abnormal and unlikely to produce viable cells. Single hepatocyte necrosis was noted after 1 week and progressed to focal tissue necrosis, principally in centrilobular areas, after 3 weeks. Clumps of leukocytes phagocytosing debris were noted in the areas of necrosis. Kupffer cell hyperplasia was evident in all livers examined. Capsular thickening was focal 1 week postinjection with lymphocytic infiltration. Three and 4 weeks postinjection the changes were diffuse. Dense fibrous tissue was present adjacent to the liver parenchyma. However, the surface collagen was very fine and contained many normal capillaries. Areas of old hemorrhage were present in the surface collagen, as was an infiltrate of lymphocytes, mast cells, plasma cells, and eosinophils. Subcapsular veins were ectatic and congested. Perisplenitis was noted 3 and 4 weeks postinjection. The capsule was thickened with fibrous tissue and infiltrated with lymphocytes. The amount of extramedullary hematopoiesis paralleled that of the controls.

Finally, IIIa was administered intraperitoneally to one rat at a reduced dose of 30 mg/kg, while a control animal was given polysorbate 80[15] (3%) in normal saline. The animals were examined at the end of a 4-week pe-

[17] Virtis homogenizer, model 45.

[18] Sigma Type H-2, Sigma Chemical Co., St. Louis, MO 63178.

riod. Perisplenitis was noted on gross examination of the test rat. Histological examination revealed focal thickenings of the splenic capsule with very fine reticulate connective tissue. Large capillaries were prominent in these areas and small hemorrhages were noted. Mast cells, some of which were degranulated, were numerous in the thickened capsule. Histologically the degree of liver change was mild with centrilobular degeneration characterized by basophilic clumping of cytoplasm, single cell necrosis with phagocytosis by invading leucocytes, and prominence of Kupffer cells.

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