(-C=N). The compound gave a strong qualitative test for nitrogen, and was characterized as **12-oxocholane azine (II)**.

Anal. Calcd. for $C_{48}H_{80}N_2$ (685.14): C, 84.14; H, 11.77; N, 4.09. Found: C, 84.28; H, 11.82; N, 3.96.

On concentration of the mother liquor and cooling, a second crop of crystals (0.31 g., 30%) was obtained, which was recrystallized from ethanol as large prisms: m.p. 178.0–180.5°; $[\alpha]D$ +60.3°; λ_{max}^{KBr} 3.1, 6.15, 6.28, 7.51, 8.62 (s), and 12.28 μ (tosylhydrazone[16]). The compound was characterized as **12-oxocholane *p*-toluenesulfonylhydrazone (I)**.

Anal. Calcd. for $C_{31}H_{50}N_2O_2S$ (514.80): C, 72.32; H, 9.79. Found: C, 72.53; H, 9.61.

Acid-Catalyzed Preparation of 12-Oxocholane Tosylhydrazone (I). A. With Hydrochloric Acid.—12-Oxocholane (30 mg.) was heated at reflux with 30 mg. of tosylhydrazine in 3 ml. of absolute ethanol containing 0.05 ml. of concentrated hydrochloric acid. The reaction was followed by t.l.c. In 10 min., a faint spot of hydrazone I appeared; in 3 hr., ketone was still present; in 5 hr., ketone had disappeared. No 12-oxocholane azine II was detected. On concentration of the solution, dense crystals (29 mg., 65%), identical with 12-oxocholane tosylhydrazone (I) according to mixture melting point, t.l.c., and infrared, separated.

B. With Acetic Acid.—An experiment identical with A, but with acetic substituted for hydrochloric acid, was carried out and monitored by t.l.c. The reaction is considerably slower than in A. Tosylhydrazone was first observed at 0.5 hr.; at 6.5 hr., ketone was still present, and azine was seen. On concentration and cooling, crystals separated which consisted of a 4:1 mixture of tosylhydrazone and azine, as estimated by t.l.c.

Hydrazine from Tosylhydrazine.—The tosylhydrazine (20 mg.) used in the previous experiment when tested with the Pesez and Petit reagent[10] showed no free hydrazine, but, when another portion in 2 ml. of absolute ethanol was refluxed for 24 hr., a strong test was obtained. When 10 mg. of 12-oxocholane was added to such a prerefluxed solution and heated at reflux, and the reaction was followed by t.l.c., a spot corresponding to the azine appeared in less than 5 min. After 90 min., the reaction was stopped; on cooling, dense microcrystals separated from the solution. These melted at 155.0–158.5° and were identical with azine II, according to R_f and infrared.

An experiment was set up identically, but with the starting reagents refluxed together. By t.l.c. no evidence of azine formation was seen even after 8 hr.

12-Oxocholane Azine from Hydrazine.—12-Oxocholane (60 mg.), refluxed in a solution containing 0.25 ml. of hydrazine hydrate (95%), 4.5 ml. of ethanol, and 0.5 ml. of acetic acid, and monitored by thin layer chromatography, showed formation of the azine within a few minutes. Reaction was complete in 35 min. On cooling, the solutions deposited dense crystals, 45 mg. (76%), identical with the azine product II, according to t.l.c., infrared, and mixture melting point.

Attempts to Convert Tosylhydrazone I to Azine II.—12-Oxocholane tosylhydrazone (30 mg.) was recovered largely unchanged either after overnight refluxing with 3 ml. of ethanol, or after 24 hr. refluxing with 30 mg. of tosylhydrazine and 3 ml. of ethanol. No azine was detected (t.l.c.) in either experiment.

Treatment of 12-Oxocholane Tosylhydrazone with Base.—Tosylhydrazone I, 100 mg., suspended in a mixture containing 230 mg. of sodium methylate and 3 ml. of ethylene glycol, was heated, and the temperature maintained at 180° for 1.5 hr. At 140°, gas evolution was observed. T.l.c. showed disappearance of the hydrazone and appearance of a major fast-running material, with R_f corresponding to that of a cholene. When processed, an oil was obtained, which appears to be a mixture of olefins.[14]

Hydrolysis of Azine by HCl.—Ketazine II (17 mg.) was suspended in 2 ml. of ethanol and 5 ml. of 10% hydrochloric acid, and refluxed for 8 hr. On cooling the solution, flaky crystals separated, which were identical with 12-oxocholane, according to melting point, t.l.c., and infrared; the yield was quantitative.

Attempted Hydrogenation of Azine II.—The ketazine II (60 mg.) in 30 ml. of ethanol was shaken with 18 mg. of platinum oxide in a Parr apparatus with hydrogen at 2 atm. pressure for 6 hr. The ketazine was recovered unchanged.

Thin Layer Chromatography.—Reactions were followed by t.l.c. using silica gel G (Merck, Darmstadt) plates. The R_f values of compounds were [development solvent, 10% ethyl acetate in ligroin (b.p. 63–70°)]: 12-oxocholane azine, 0.95; 12-oxocholane, 0.82; 12-oxocholane tosylhydrazone, 0.38; tosylhydrazine, 0.00. The spray used was ethanol–sulfuric acid–vanillin.[17]

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Reactions of Potassium *t*-Butoxide in Dimethyl Sulfoxide. IV.[1] With Primary Tosylates and Halides*

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When treated with potassium *t*-butoxide in dimethyl sulfoxide (KtBD)[2] at room temperature, 24-cholanyl tosylate yielded predominantly the *t*-butyl ether, as might be expected from previous work.[3] 24-Chlorocholane, however, surprisingly reacted with KtBD to give mainly the elimination product, 23-cholene. The strikingly unexpected difference between tosylate and corresponding chloride led us to investigate further reactions on 24-iodocholane and on selected aliphatic derivatives.

Experimental results of these studies, summarized in Table I, indicate that the difference between primary tosylates and primary halides in reaction with KtBD is a general one: tosylates give predominantly substitution products; halides, chiefly products of elimination. The one exception encountered was perforce a methyl halide.

TABLE I

PRODUCTS OF REACTION OF POTASSIUM *t*-BUTOXIDE IN DIMETHYL SULFOXIDE WITH PRIMARY TOSYLATES AND HALIDES AT ROOM TEMPERATURE

Compd.	Reaction time, min.	Yield of products—Ene (%)	Ether (%)
24-Cholanyl tosylate	30	*a* (21)	*b* (78)
chloride	15	*a* (79)	*b* (21)
iodide	<1	*a* (73)	*b* (17)
n-Octadecyl tosylate	<5	*c* (25)	*d* (71)
n-Octadecyl chloride	5	*c* (86)	*d* (14)
iodide	<1	*c* (90)	*d* (10)
n-Octyl tosylate[e]	...	*e* (16[f])	*g* (49[f])
iodide	*h*	*e* (90)	*g* (10)
methyl iodide	*h*	...	*i* (78)

[a] 23-Cholene. [b] 24-Cholanyl *t*-butyl ether. [c] 1-Octadecene. [d] *n*-Octadecyl *t*-butyl ether. [e] 1-Octene. [f] From ref. 3b. [g] *n*-Octyl *t*-butyl ether. [h] Reaction could not be monitored by t.l.c. [i] Methyl *t*-butyl ether.

During the final stages of this work prior to preparation of this paper, Veeravagu, Arnold, and Eigenmann[4] reported very much slower reactions in which primary

* To Professor Louis F. Fieser.

(1) Part III: *Tetrahedron Letters*, 2969 (1964).

(2) KtBD has been proposed as an abbreviation for this reagent by F. C. Chang and N. F. Wood [*Steroids*, **4**, 55 (1964)].

(3) (a) R. T. Blickenstaff and F. C. Chang, *J. Am. Chem. Soc.*, **80**, 2726 (1958); (b) C. H. Snyder and A. R. Soto, *J. Org. Chem.*, **29**, 742 (1964).

(4) P. Veeravagu, R. T. Arnold, and E. W. Eigenmann, *J. Am. Chem. Soc.*, **86**, 3072 (1964).

tosylates and bromides, when heated at reflux with potassium *t*-butoxide in *t*-butyl alcohol, gave results essentially similar to ours. Since their comments and discussion on the surprising difference between halides and *p*-toluenesulfonates are essentially applicable to our study, and since we have no satisfactory explanation for the difference to offer at this time, we will merely call further attention to the versatility of KtBD as a reagent in both substitution and elimination reactions.

Experimental[5]

Reactions with KtBD.—KtBD reagent used in this work was a 1 *N* solution, prepared from potassium *t*-butoxide (M. S. A. Corp., Callery, Pa.) and dimethyl sulfoxide (J. T. Baker, reagent grade), dried over Molecular Sieves (Linde Co., 13X type, 0.0625-in. pellets). Compounds, with the exception of methyl iodide and 1-iodooctane, were dissolved in benzene before treatment with the reagent. In the case of the tosylates, the reaction mixture turned a dark green; with halides, there was little development of color. Reactions involving the cholane and octadecane derivatives were carried out as given for 24-tosyloxycholane and 1-octadecyl chloride, respectively. Where possible, reactions were monitored by thin layer chromatography (t.l.c.). Samples from the reaction mixture were spotted and reaction immediately was quenched in the layer by addition of 1 *N* hydrochloric acid.

A. With 24-Tosyloxycholane.—24-Tosyloxycholane[6] (132 mg., 0.264 mmole), dissolved in 2 ml. of benzene, was treated with 2 ml. of 1 *N* KtBD. The total reaction mixture was chromatographed on a column of Florisil[7] (60–100 mesh, 24 g.). Both fractions eluted with ligroin[8] and with ligroin–ether (4:1) yielded crystalline material, weighing 10 mg. (21%) and 83 mg. (78%), respectively. The former, 23-cholene, recrystallized from methanol, had m.p. 99.0–100.4°; $[\alpha]_{589}$ +10.5°, $[\alpha]_{546}$ +11.9°, and $[\alpha]_{405}$ +18.9°; $\lambda_{max}^{CS_2}$ 3.24, 6.13, 10.10, and 10.99 μ (vinyl).

Anal. Calcd. for $C_{24}H_{40}$ (328.56): C, 87.64; H, 12.24. Found: C, 87.73; H, 12.28.

The latter, 24-*t*-butoxycholane, also recrystallized from methanol, had m.p. 95.0–97.0°; $[\alpha]_{589}$ +20.0°, $[\alpha]_{546}$ +23.2°, and $[\alpha]_{405}$ +44.6°; $\lambda_{max}^{CS_2}$ 7.37, 8.00, 8.14, 8.37, 9.28, and 9.83 μ[9] (lit.[10] m.p. 95.0–95.5°, $[\alpha]_{589}$ +20.7°, $\nu_{max}^{CHCl_3}$ 1074 cm.$^{-1}$).

23-Cholene and **24-*t*-butoxycholane** obtained in the experiments with 24-chlorocholane[11] and 24-iodocholane[12] were identical with the corresponding products from the tosylate, according to melting point, infrared spectra, and R_f comparisons.

B. With *n*-Octadecyl Chloride.[13]—To the chloro compound (1.44 g., 5 mmoles) dissolved in 10 ml. of benzene was added 10 ml. of 1 *N* KtBD. The clear solution became cloudy and slightly warm. The entire reaction mixture was chromatographed on Florisil (60–100 mesh, 50 g.). The first 300 ml. of ligroin eluted an oil, which on rechromatography yielded, in the ligroin fractions, 1.08 g. (86%) of 1-octadecene, $n^{25}D$ 1.4421 (lit.[14] n_D 1.4429), $\lambda_{max}^{CS_2}$ 3.25, 6.13, 10.10, and 10.99 μ (vinyl); and, in the ligroin–ether (1:1) fractions, 0.23 g. (14%) of *n*-octadecyl *t*-butyl ether,[15] $n^{25}D$ 1.4396, $\lambda_{max}^{CS_2}$ 7.35, 7.96, 8.12, 8.34, 9.24, 9.80, 11.40, and 13.88 μ.[9]

Anal. Calcd. for $C_{22}H_{46}O$ (326.59): C, 80.89; H, 14.20. Found: C, 81.18; H, 13.92.

Both products were homogeneous by t.l.c.

1-Octadecene and *n*-octadecyl *t*-butyl ether obtained from the reactions involving *n*-octadecyl tosylate[16] and iodide[17] were identical with the corresponding products obtained from the chloride, according to t.l.c. and infrared spectra comparisons.

C. With *n*-Octyl Iodide.[18]—*n*-Octyl iodide (6 g., 25 mmoles) was treated with 50 ml. of 1 *N* KtBD. The mixture, with the halide forming a layer at the bottom of the reaction vessel, became warm immediately and, when shaken, reacted rapidly to yield an oily top layer. After 0.5 hr., 5 ml. of ligroin and ice-cold water were added. The whole was shaken to saturation with sodium chloride, and the hydrocarbon phase was separated. The aqueous layer was shaken with three more 5-ml. portions of ligroin. The combined extracts were washed with brine, dried, and diluted to 25 ml. with ligroin. A portion was evaporated to an oil, which was separated by preparative gas–liquid chromatography (Aerograph, A-700), using a 20 ft. × 0.375 in. 10% diethylene glycol succinate on Chromosorb W, 60–80 mesh, column, injector temperature 202°, column temperature 125°, flow rate 200 cc./min. Retention times were for the olefin, 2.1 min.; for the ether, 3.5 min.; and for the iodide, 5.5 min.

1-Octene: $n^{20}D$ 1.4093 (lit.[19] n_D 1.4088); $\lambda_{max}^{CS_2}$ 3.23, 6.10, 10.07, and 10.99 μ (vinyl).

1-Octyl *t*-butyl ether: $n^{20}D$ 1.4184; $\lambda_{max}^{CS_2}$ 7.35, 7.96, 8.12, 8.34, 9.24, 9.80, 11.40, and 13.88 μ [lit. n_D 1.4184,[3b] ν 2925, 1470–1465, 1395, 1365, 1250–1200 (incompletely resolved multiplet), 1080, 880, and 730 cm.$^{-1}$][20].

Using these as standards, the mixture was estimated by further g.l.c. to consist of 90% 1-octene and 10% *n*-octyl *t*-butyl ether.

D. With Methyl Iodide.—To a stirred slurry of potassium *t*-butoxide (13 g.) in dimethyl sulfoxide (40 ml.) was slowly added methyl iodide (6.25 ml., 0.1 mole) with intermittent cooling of the reaction mixture. Stirring was continued for 10 min. after completion of the addition, and ice and water were added to bring the total volume to about 200 ml. A layer of methyl *t*-butyl ether formed on the surface of the aqueous solution. After saturation of the aqueous layer with sodium chloride, the ether layer was separated, washed with saturated sodium chloride, and dried over magnesium sulfate; weight, 4.52 g. The mother liquor was extracted with three 12.5-ml. portions of *n*-butyl ether; the combined extracts were washed with brine and distilled over sodium. The lowest boiling fraction (60–65°) of methyl *t*-butyl ether was collected; weight, 2.82 g. The total product was examined by g.l.c. and found to contain about 6% *t*-butyl alcohol. A 20 ft. × 0.375 in. 10% diethylene glycol succinate column at 77° was used with a flow rate of 200 cc./min. Retention times for methyl *t*-butyl ether and *t*-butyl alcohol were 2.5 and 3.5 min., respectively. The estimated yield of ether was 78%. Several washings of the product with water removed the *t*-butyl alcohol and gave material with b.p. 56.0–56.5° and $n^{25}D$ 1.3669 (lit.[21] b.p. 55°, $n^{25}D$ 1.3667).

Thin layer chromatography was performed on silica gel G (Merck, Darmstadt), using sulfuric acid–ethanol–vanillin spray.[22] The R_f values for the cholane derivatives were (for chloroform solvent) 24-cholanol, 0.17; 24-cholanyl tosylate, 0.61; 24-cholanyl *t*-butyl ether, 0.67; and 23-cholene, 0.84, and (for ligroin solvent) 24-cholanyl *t*-butyl ether, 0.01; 24-cholanyl chloride, 0.56; 24-cholanyl iodide, 0.60; and 23-cholene, 0.73. The R_f values for aliphatic derivatives were (for chloroform solvent) 1-octadecanol, 0.19; *n*-octadecyl tosylate, 0.63; *n*-octadecyl *t*-butyl ether, 0.74; and 1-octadecene, 0.87, and (for ligroin solvent) *n*-octadecyl *t*-butyl ether, 0.05; *n*-octadecyl chloride, 0.63; *n*-octadecyl iodide, 0.67; and 1-octadecene, 0.76.

(5) Microanalyses were performed by A. Bernhardt, Mülheim, Germany. Melting points were determined by electrical micro hot stage and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Infracord No. 127.

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(7) Product of the Floridin Co.

(8) Ligroin used in this work was Skellysolve B (Skelly Oil Co.), b.p. 63–70°, further purified by sulfuric acid treatment and distillation.

(9) The series of bands of characteristic relative intensities, at 7.93, 8.12, 8.33, 9.24, and 9.79 μ, is common to all four *t*-butyl ethers reported in this work, as well as to 3β-cholestanyl *t*-butyl ether. We are not aware of any previous infrared characterization of a *t*-butyl ether.

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An Experimental Demonstration of the Nuclear Magnetic Resonance Assignments in the 6,7-Dimethylisoalloxazine Nucleus*

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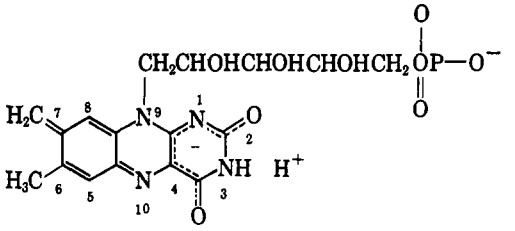
We have observed (Figure 1) that the amplitude of the downfield methyl peak in the n.m.r spectrum of riboflavin 5′-phosphate (FMN) decreases when the material is heated at 90–95° in D_2O solution buffered at pH 6.8 to 6.9.[2] This is the optimum pH for effecting the reaction (approximate rate constant 2.4×10^{-4} sec.$^{-1}$). At higher pH (7.5) more rapid decomposition complicates the result, and in unbuffered solution (pH 5.8), we have been unable to observe any reaction during comparable heating periods. Heating periods of up to 3 hr. fail to give any evidence of a decrease in amplitude of any other peak.

We attribute the selective decrease in amplitude of the downfield methyl peak to an exchange of the C-7-methyl protons with solvent through the intermediacy



1

of **1**. The facile exchange is then understandable since the possibility of forming a highly delocalized anion undoubtedly helps compensate for the loss of the resonance stabilization associated with the aromatic ring. No completely conjugated, delocalized structure can be invoked which might account for such a facile exchange occurring at the C-6 methyl. On chemical grounds then, the assignment of the downfield methyl peak to the C-7 methyl seems supportable, but we can also offer the following evidence that this assignment is indeed correct.

Comparison of the spectra of lumiflavin (6,7,9-trimethylisoalloxazine, Figure 2) and its 6-ethyl analog and of *o*-xylene and *o*-ethyltoluene (in CS_2)[3] shows that

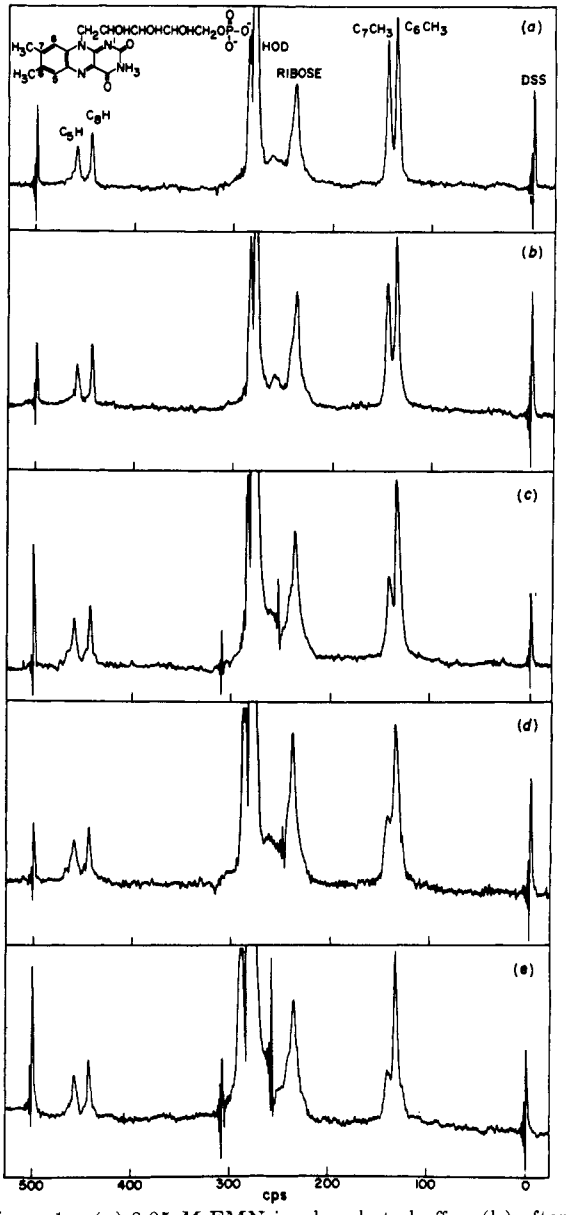


Figure 1.—(a) 0.05 *M* FMN in phosphate buffer, (b) after 55 min., at 92–93°, (c) after 1.5 hr., (d) after 2 hr., (e) after 3 hr. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate was added as internal standard. Symmetrically spaced lines near the water peak in some spectra are spinning side bands.

in both these series, conversion of methyl to ethyl results in the appearance of the methylene (quartet) resonance 19 to 21 c.p.s. downfield from the shift of its methyl precursor. Also there is no shift (to within 2 c.p.s.) of the unsubstituted methyl resonance. Thus the downfield peak at 138 c.p.s. in the isoalloxazines must be assigned to the C-7 methyl.

A dimerization of riboflavin under basic conditions, considered[4] to occur through the C-7 methyls, has previously been demonstrated and attributed to the intermediacy of a methide structure similar to **1**. The ready accessibility of **1** in essentially neutral solution is, however, an interesting aspect of the chemistry of these materials.

Based on the comparison of the spectra of lumiflavin and 8-deuteriolumiflavin, we assign the upfield aromatic

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(2) Meter readings, without correction for differences in activity in D_2O.

(3) For 0.1 *M* solutions (CS_2) the methyl shifts are *o*-xylene, −159 c.p.s., and *o*-ethyltoluene, −161 c.p.s. The CH_2 quartets are centered at −180.5 c.p.s. for *o*-ethyltoluene and −183 c.p.s. for ethylbenzene. The shifts are expressed relative to external hexamethyldisiloxane.

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