TABLE I

Expt. no.	41	24	25	40	45
Solvent, mole		Nitromethane		Nitrobenzene	
	0.21	0.92	0.08	0.29	0.31
Aromatic	Benzene	Toluene	Toluene	Benzene	Toluene
Products[a]					
t-Butyl-	6.7	15.5	24.9	4.3	..
p-*t*-Octyl	..	62.6	44.6	..	80.5
m-*t*-Octyl	..	...	4.9	..	4.2
Octyl-	28.2	8.4	18.3	0.6	..
Di-*t*-butyl	13.0	...	...	0.4	..
Dodecyl	..	...	9.2	..	..
C_{12}-[b]	28.0	...	...	0.9	..
Polymer	4.8	15.3	...	73.5	..
Olefin consumed	80.7	101.8	101.9	79.7	84.7

[a] Wt. % of diisobutene consumed ending up as substituent group or polymer. [b] Mixture of dodecyl, octyl–butyl, tributyl.

***p*-*t*-Octylbenzoic Acid, 4-(1,1,3,3-Tetramethylbutyl)-benzoic Acid.**—Oxidation of 8 g. of the *t*-octyltoluene with 12 g. of potassium permanganate in 65 g. of pyridine at 88° for 7 hours, following the general procedure of Nightingale and Janes,[8] yielded 4 g. of colorless platelets after recrystallization from isoöctane; m.p. after two recrystallizations, 158.5–159.5°.

Anal. Calcd. for $C_{15}H_{22}O$: C, 76.88; H, 9.46; neut. equiv., 234.3. Found: C, 76.87, 76.98; H, 9.52, 9.51; neut. equiv., 236, 236.6.

We have extended this study of the intact alkylation of aromatics to include various isobutene polymers and a variety of catalysts and operating conditions. A complete report of these results will be made at a later date.

(8) D. Nightingale and J. R. Janes, THIS JOURNAL, **66**, 155 (1944).

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Effect of Ultraviolet Light on Steroids during Paper Chromatography[1]

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Since the first description of the paper chromatographic separation and purification of corticosteroids[2] this technique has been rapidly adopted by many workers in the field.[3] Two of the difficulties often encountered in the application of this technique are the frequent inability to measure steroids quantitatively following chromatography, and to obtain from paper chromatograms samples of steroids (despite their apparent crystalline purity) completely free from contaminants which can best be described by their appearance in subsequent chromatograms of the sample in question, as highly polar material which does not migrate from the starting line. This latter problem is particularly noticeable in dealing with radioactive steroids containing carbon-14 (and has been observed by others working with C^{14}-desoxycorticosterone and progesterone[4]). The occurrence of this immobile material persists despite the distance the steroid has traversed from the starting line in earlier chromatograms, and its intensity has been related to the *interval of time between the removal of the chromatogram for drying and the subsequent elution* of the steroid. The two aspects of this problem have been investigated independently in these laboratories and the following preliminary observations are presented in order to acquaint workers employing these techniques with what is at best an aggravating phenomenon.

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(2) (a) A. Zaffaroni, R. B. Burton and E. H. Keutman, *Science*, **111**, 6 (1950); (b) R. B. Burton, A. Zaffaroni and E. H. Keutman, *J. Biol. Chem.*, **188**, 763 (1951).

(3) (a) D. Kritchevsky and M. Calvin, THIS JOURNAL, **72**, 4330 (1950); (b) O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **34**, 108 (1951); (c) T. H. Kritchevsky and A. Tiselius, *Science*, **114**, 299 (1951); (d) I. E. Bush, *Biochem. J.*, **50**, 370 (1952); (e) R. Neher and A. Wettstein, *Helv. Chim. Acta*, **35**, 276 (1952); (f) C. D. Kochakian and G. Stidworthy, *J. Biol. Chem.*, **199**, 607 (1952); (g) L. R. Axelrod, *ibid.*, **201**, 59 (1953); (h) K. Savard, *ibid.*, **202**, 357 (1953).

Experimental

Effect of Ultraviolet Light on Testosterone-3-C^{14}.—Six milligrams of testosterone-3-C^{14} (49,000 c./min./mg.)[5] was chromatographed on a sheet of filter paper (8 × 50 cm.) in the solvent system ligroin–propylene glycol[2b] for 48 hours; the distribution of radioactivity and the area occupied by the testosterone are shown in Fig. 1a. After the usual interval (16–24 hours) allowed for drying in air, the area of the chromatogram containing the testosterone was cut out and eluted with methanol; the testosterone was reapplied to a second sheet of filter paper and chromatographed in ligroin–propylene glycol for 48 hours. Again after drying, the testosterone was located and the distribution of radioactivity determined; these are shown in Fig. 1b. The small amount of polar material at the starting line appeared in the expected manner. The chromatogram was exposed, on both sides, to the irradiation of an ultraviolet source (Mineralite, Ultraviolet Products, Pasadena, California) at a distance of 12 cm. for 2 hours. The area of the testosterone was then eluted and chromatographed on a third sheet of filter paper for 48 hours in ligroin–propylene glycol. The distribution of radioactivity in this chromatogram is shown in Fig. 1c, together with the colored areas which appeared when the strip was exposed to the Zimmermann reagent. As can be seen, there is a major increase in the amount of polar material at the starting line with a corresponding decrease in the area and radioactivity of the testosterone zone. Even more impressive is the appearance in serious concentration of at least *five* additional steroid areas, all of which gave positive (violet and blue) color reactions with the Zimmermann reagent. Interpretation of the location of these zones, as well as the colors they gave with the Zimmermann reagent[2b] suggest that the following reactions had occurred: saturation of the 4,5-double bond, or its migration from that position in conjugation with the 3-ketone, or both; formation of 17-ketosteroids (violet Zimmermann color) indicating oxidation of the 17β-hydroxyl group.

Recovery of Steroids from Irradiated Chromatograms.—In the following experiments 200 γ of steroid was applied to each of several 1-cm. strips of filter paper and chromatographed in ligroin–propylene glycol for the time required to move the steroid in question to approximately half the length of the paper strip (24 hours for testosterone, 16 hours for Δ^4-androstene-3,17-dione, 8 hours for progesterone). A completed chromatogram containing each steroid was dried in an air-oven at 60° for 30 minutes, while duplicate chromatograms were exposed to the Mineralite source of ultraviolet light for 2 hours. The steroid areas were then eluted with methanol and the absorption at 240 mμ was measured in methanol in the Beckman spectrophotometer. Correction was made in all these readings for absorbing material by the elution from each chromatogram of an area of paper equal to that occupied by the steroid being measured; this was accomplished by utilizing an eluate of the non-steroid area of the chromatogram as blank. The results are listed in Table I, along with the results obtained with cortisone after chromatography in toluene–propylene glycol[2b] for 96 hours.

Evidence for the destruction of the α,β-unsaturated ketone structure of the steroids by ultraviolet light was ob-

(4) R. D. H. Heard, private communication.

(5) Prepared by Dr. Marcel Gut.

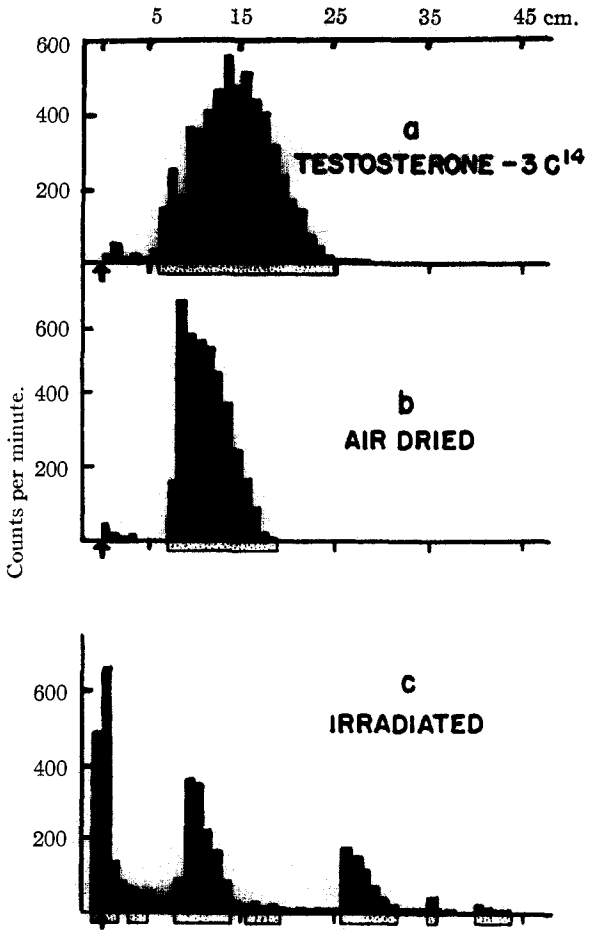

Fig. 1.—Chromatography in ligroin–propylene glycol (48 hr.): a, testosterone-3-C^{14}; b, material from (a) dried in air; c, material from (b) exposed to ultraviolet light (2 hours). Radioactivity determined in flow-gas counter upon 1 × 2 cm. strips of paper chromatogram. Stippled areas indicate positive reaction with Zimmermann reagent.

TABLE I

Steroid	Amt. added, γ	Oven dried, min.	Ultraviolet radiation Distance Exposure, hr.	Distance from light, in.	Steroid recovered, γ
Testosterone	200	30			199
	200	30			200
	200	30			196
	200	30			198
	200		2	8	145
	200		2	8	156
	200		2	2	10
	200		2	2	32
Δ^4-Androstenedione	200	30			198
	200	30			196
	200		2	4	78
	200		2	4	96
Progesterone	200	30			193
	200	30			199
	200		2	6	133
	200		2	6	98
Cortisone	200	30			187
	200	30			183
	200		2	3	69
	200		2	3	98

tained by the examination of the ultraviolet absorption spectra of the steroid eluates obtained above. Fig. 2 shows the absorption spectra obtained with testosterone, androstenedione and progesterone, following oven drying, and following exposure to ultraviolet light.

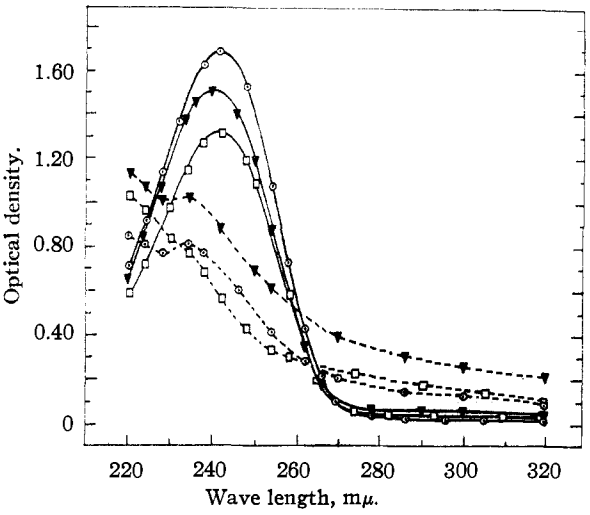

Fig. 2.—Ultraviolet absorption spectra of ketosteroids eluted from heat-dried chromatograms (solid lines), and ultraviolet irradiated chromatograms (broken lines), 200 γ of ketosteroid in 8–10 ml. in methanol: progesterone ⊙; Δ^4-androstene-3,17-dione ▼; testosterone □.

That the effect of ultraviolet light upon steroids is not confined to those steroids possessing the α,β-unsaturated ketone system, was illustrated by the exposure of chromatograms containing 200 γ of androsterone and dehydroepiandrosterone to ultraviolet light under identical conditions, as

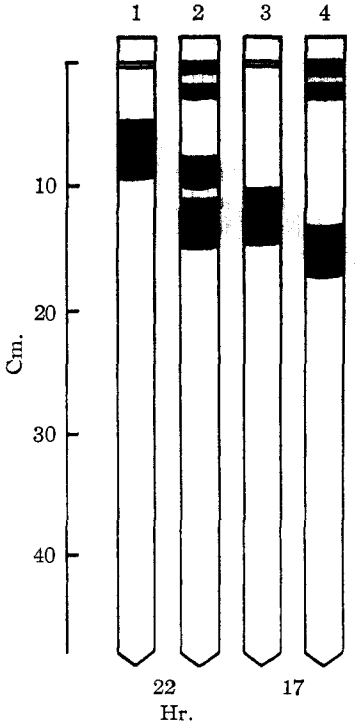

Fig. 3.—Effect of ultraviolet light upon steroids: standard dehydroepiandrosterone, 50 γ, strip 1; irradiated, strip 2; standard androsterone 50 γ, strip 3; irradiated, strip 4. Chromatography in ligroin–propylene glycol; steroids visualized by Zimmermann reagent.

described above. Elution of the exposed areas of these chromatograms and rechromatography in ligroin–propylene glycol and exposure of the strip to the Zimmermann reagent revealed the presence of additional steroid presumed areas, apart from those of the unchanged steroids. Several such chromatograms are illustrated in Fig. 3.

Discussion

The so-called "decomposition" of steroids when exposed to oxygen (or air) and to various forms of radiant energy (ultraviolet light, X-rays) has been recognized for some time. That these effects on the stability of steroids occur during paper chromatography is not unexpected since the steroid is usually spread over a considerably large area which affords maximal conditions of exposure to light and to air. The observations reported here indicate that ultraviolet light (present in artificial light and sunlight) can effect chemical changes in steroid samples during the drying of a paper chromatogram. Other occasions of likely exposure of a steroid preparation to light and to air can and do occur in the course of the various manipulations inherent in the paper chromatographic technique (application of sample to the starting line, development of the chromatogram). It has been noted that α,β-unsaturated ketosteroids undergo destruction[6] when overexposed in the Haines–Drake paper chromatogram ultraviolet scanner.[7] Chromatographically homogeneous desoxycorticosterone and progesterone (containing C^{14}) invariably give rise to immobile material (remaining at the starting line) when rechromatographed on paper[4] in toluene–propylene glycol, as has been the case for the testosterone-3-C^{14} in our hands. The appearance of the immobile material in the initial paper chromatogram of the synthetic C^{14}-testosterone (Fig. 1a) would suggest that the apparent chemical change had occurred prior to the development of the chromatogram. Whether this decomposition had occurred during the application of the sample to the paper, or during the development of the chromatogram, or whether it was due to autoradiation as recently reported for highly radioactive cholesterol[8] cannot be determined from the present data. As a precautionary measure it has become the practice in these laboratories[9] to dry the chromatograms as rapidly as possible in the absence of light at temperatures not exceeding 50°. This method has provided adequate chemical recoveries from paper chromatograms of α,β-unsaturated ketosteroids (Table I) and urinary 17-ketosteroids.[10] Where heat-labile corticosteroids are concerned, it has been found best to restrict the drying period to less than one hour at room temperature, shielded from direct sunlight or fluorescing lights; the residual solvent (propylene glycol, formamide) is later removed from the eluted steroid preparation *in vacuo* over phosphorus pentoxide, or by partition between ether or methylene dichloride and water. With C^{14}-containing steroids this method reduces but does not entirely prevent the persistent reappearance of the immobile starting line material shown in Fig. 1a and b, and more recently by more sensitive autoradiographic methods.

(6) D. H. Peterson, private communication.

(7) W. J. Haines and N. A. Drake, *Federation Proc.*, **9**, 180 (1950).

(8) W. G. Dauben and I. L. Chaikoff in B. M. Talbot, *et al.*, THIS JOURNAL, **75**, 1867 (1953).

(9) K. Savard, S. Burstein, H. Rosenkrantz and R. I. Dorfman, *J. Biol. Chem.*, **202**, 717 (1953).

(10) B. Rubin, R. I. Dorfman and G. Pincus, *ibid.*, **75**, 629 (1953).

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The Diels–Alder Reaction of 2-Vinylthiophene

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Furan, isobenzofurans and α-pyrone are the only heterocyclic compounds known to add maleic anhydride.[2] Furan undergoes the Diels–Alder reaction with extraordinary ease; thiophene, however, does not although it does possess an apparent conjugated diene system. Similarly, benzene and naphthalene do not undergo this reaction, but vinylbenzene and 1-vinylnaphthalene form adducts with maleic anhydride and other dienophiles.[3] It was expected that the thiophene analog of these compounds might also undergo the Diels–Alder reaction with the formation of a partially hydrogenated thianaphthene derivative. Since the inception of this investigation, it has been reported that thienylcycloalkenes[4] have successfully undergone the Diels–Alder reaction with maleic anhydride.

2-Vinylthiophene with maleic anhydride in dry benzene was warmed on a steam-bath for 4 hours to obtain the adduct. When the reaction mixture was allowed to stand for long periods, after heating (overnight), the yield of adduct was decreased and that of copolymer increased. The yield of adduct was much lower when the reaction was allowed to proceed at room temperature.

After the separation of the copolymer and polymerized vinylthiophene, the adduct was hydrolyzed to a dicarboxylic acid; elemental analysis and neutral equivalent indicated this acid to be tetrahydrothianaphthenedicarboxylic acid. The latter compound melted over a range of temperature, indicating a mixture. The acid exhibited partial solubility in ethyl acetate; elemental analysis of the ethyl acetate-soluble acid and the insoluble acid gave identical results. It was assumed that these are *cis–trans* isomers arising from the hydrolysis of the acid anhydride. The higher melting acid, insoluble in ethyl acetate, was isomerized to the lower melting acid by the method of Bachmann.[5] The lower melting acid was unaffected by this method of isomerization.

In order to establish the structure of these acids, they were converted to thianaphthene by dehydrogenation with sulfur, followed by decarboxylation with barium hydroxide.

(1) Based on a portion of the thesis submitted by J. F. Scully in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Fordham University.

(2) M. C. Kloetzel, "Organic Reactions," Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 6.

(3) N. C. Deno, Univ. Microfilm, Publ. 1161, Ann Arbor, Michigan, 1948.

(4) J. Szmuszkovicz and E. J. Modest, THIS JOURNAL, **72**, 571 (1950).

(5) W. E. Bachmann, *ibid.*, **70**, 1462 (1948).