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.

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The Diels–Alder Reaction of 2-Vinylthiophene

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RECEIVED AUGUST 21, 1953

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.

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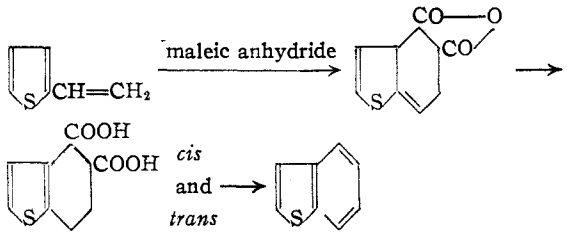

Experimental[6]

2-Thienylethanol.—The Grignard reagent from 130 g. (0.62 mole) of 2-iodothiophene[7] was prepared by the usual procedure and the reagent transferred by nitrogen pressure to a 1-liter, 3-neck, round-bottomed flask, equipped with a mercury-sealed stirrer, dropping funnel and thermometer and immersed in an ice–salt mixture. A solution of 30 g. of ethylene oxide in 100 ml. of dry benzene was added as quickly as possible, while maintaining the temperature at −10°. The reaction was very vigorous and exothermic but was easily controlled by regulating the flow through the dropping funnel and stirring at high speeds. The temperature was allowed to rise to room temperature and the mixture stand for one hour. It was poured into a dilute solution of hydrochloric acid and the ether–benzene layer separated and dried over anhydrous sodium sulfate. The ether and benzene were removed and the product distilled at reduced pressure to obtain 40 g. (50%) of 2-thienylethanol,[8] a water-white liquid, b.p. 99–100° (7 mm.).

2-Vinylthiophene was prepared by the dehydration of 2-thienylethanol,[8] and also by the method of Emerson and Patrick.[9]

Adduct Formation.—2-Vinylthiophene, 60 g. (0.55 mole), and 53.9 g. of maleic anhydride in 150 ml. of dry benzene were gently refluxed for 4 hours on a steam-bath. The hot benzene solution was decanted from the solid residue that had separated during the refluxing; the residue was extracted with 100 ml. of hot benzene. The combined benzene solutions were evaporated at reduced pressure to obtain the adduct.

The adduct was dissolved in 30% potassium hydroxide, solution being effected by gentle warming on the steam-bath. The resultant solution was cooled in an ice-bath and carefully acidified with 6 *N* hydrochloric acid. The precipitated acid was filtered and dried. Recrystallization from methanol gave 45 g. (35%) of 4,5,6,7-tetrahydrothianaphthene-4,5-dicarboxylic acid, m.p. 180–185°; neut. equiv. calcd. 113, found 112.

Anal. Calcd. for $C_{10}H_{10}O_4S$: C, 53.10; H, 4.43. Found: C, 53.14; H, 4.38.

The acid was partially dissolved in 200 ml. of hot ethyl acetate; the volume of the solvent was reduced to 150 ml. On cooling, a white crystalline precipitate separated and was filtered. Evaporation of the filtrate to 75 ml. yielded a second crop of crystals. The total yield was 22 g., which was recrystallized from methanol, m.p. 188–189°.

Anal. Calcd. for $C_{10}H_{10}O_4S$: C, 53.10; H, 4.43. Found: C, 52.92; H, 4.16.

The ethyl acetate-insoluble acid was recrystallized from methanol to obtain 20 g. of white needles, m.p. 194–195°.

Anal. Calcd. for $C_{10}H_{10}O_4S$: C, 53.10; H, 4.43. Found: C, 52.93; H, 4.19.

Isomerization.—Three grams of the acid (m.p. 188–189°) was dissolved in 50 ml. of glacial acetic acid and the solution was saturated with dry hydrogen chloride gas. After the mixture had boiled for 4 hours under a continuous stream of hydrogen chloride, the gas was removed by boiling. The acetic acid solution was concentrated to half its volume after which dilution with water gave the unchanged acid.

One gram of the acid (m.p. 194–195°) was treated with dry hydrogen chloride gas in glacial acetic acid as described above. The isolated product was recrystallized from ethyl acetate, m.p. 186–187.5°. Mixed melting point with the acid (m.p. 188–189°) showed no depression.

Structure Proof of Adduct.—The acid (m.p. 188–189°) and 1.5 g. of sulfur were placed in a 50-ml. claisen flask, fitted with a gas trap, containing silver nitrate solution. The flask was immersed in an oil-bath and heated to 200°, where the mixture turned dark brown and began to effervesce, with the evolution of hydrogen sulfide gas. The gas was evolved rapidly at 205°; heating was continued until the evolution stopped, as evidenced by the formation of silver sulfide.

To the aromatized anhydride was added 15 g. of barium hydroxide and the mixture distilled to yield a yellow oil, which was steam distilled from a solution of potassium hydroxide. The yellow oil was identified as thianaphthene through the picrate, m.p. 145–147°. Mixed melting point with an authentic sample of thianaphthene picrate showed no depression.

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Low Concentration Chemistry. VI. Some Properties of Tracer Gold in Solution

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RECEIVED JULY 16, 1953

The purpose of these experiments was to investigate the radiocolloidal behavior, solvent extraction properties and adsorption characteristics of gold in very low concentration solutions using gold-199 as a tracer.

Experimental

Preparation of Gold Solutions.—Gold-199 was obtained from the Oak Ridge National Laboratory in irradiated platinum foil. The nuclide has a half-life of 3.3 days,[1] decaying with the emission of 0.45-, 0.29- and 0.24-mev. β-particles and 0.21-, 0.16- and 0.05-mev. γ-rays.[2] A modification of the method of Gile, Garrison and Hamilton[3] was used to separate the gold and then hydrochloric acid solutions of the gold were prepared. Spectrographic analysis of a piece of the platinum foil and the microchemical test of Hahn[4] indicated that the gold solutions were more dilute than 10^{-8} *M*.

Adjustments of *p*H.—The various solutions were adjusted to desired *p*H values by adding either sodium hydroxide or hydrochloric acid solution. Measurements of *p*H were made on a Beckman Model G-2 Glass Electrode *p*H Meter using microelectrodes.

Sample Preparation.—Samples were taken with a 0.100-ml. micropipet and syringe. These samples were placed on copper planchets or glass cups and evaporated to dryness under a heat lamp.

Radioactivity Apparatus.—Radioactivity measurements were made with a 3.5 mg./cm.^{2} end-windows, halogen-quenched G–M tube attached to a Tracerlab SC-2A Scaler. All samples were counted for a sufficiently long time to give a standard deviation equal to or less than 1%.

Filtration.—Five-ml. portions of the radiogold solution originally 0.01 *N* in hydrochloric acid were adjusted to desired *p*H values and were filtered through Whatman No. 50 filter paper. Samples were taken before and after filtration, the differences in radioactivities being used to determine the percentage removal.

Centrifugation.—Portions of the radiogold solution originally 0.01 *N* in hydrochloric acid were adjusted to desired *p*H values, placed in centrifuge tubes holding about 0.7 ml., and allowed to stand until adsorption equilibrium had been reached. This latter process required a time of about an

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