described in the previous section. Radioactive leucine (5.0 mg.) and D-glucose (100 mg.) were dissolved in a few drops of 0.1 *M* acetate buffer, *p*H 5, in a small tube, which was then heated at approximately 80° for one hour in a water-bath. The semi-dry mixture, *still completely colorless*, was dissolved in 5.0 ml. 1.5 *N* hydrochloric acid and chromatographed on a cold Dowex-50 ion-exchange resin column. The result is shown in Fig. 2. The pattern is very similar to that obtained with liver extracts (Fig. 1). The radioactivity in the new compound amounted to 14% of radioactivity added as leucine, and only about 50% of the total radioactivity was recovered. Paper chromatographic analysis gave results similar to those already described for the compound isolated from liver extracts.

**The Reaction between D-Ribose and Radioactive Leucine.**—A mixture of 100 mg. of D-ribose and 5.0 mg. carboxyl-$C^{14}$-L-leucine were heated at *p*H 5 in a "semi-dry" condition as described for the glucose–leucine mixture. A great deal of browning took place, in contrast to the glucose–leucine reaction mixture. The chromatographic analysis is shown in Fig. 3. It should be noted that only a small amount, if any, free leucine remained. The brown colored products emerged at the "front" and then gradually diminished. A number of well defined radioactive peaks appeared but these products have not been analyzed further. The greater reactivity of ribose as compared to glucose is in accord with the observation of other investigators.[22]

**Acknowledgment.**—The financial support of Eli Lilly and Company is gratefully acknowledged.

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## Absorption Spectra of Fuming Sulfuric Acid Chromogens Obtained from the Estrogens and Other Steroid Compounds[1]

BY LEONARD R. AXELROD

RECEIVED JUNE 30, 1953

Concentrated sulfuric acid has been observed to form chromogens with steroids which give absorption spectra different for each compound.[2] These absorption spectra have been utilized to aid in the qualitative identification of steroid metabolites.[3,4] Fuming sulfuric acid has now been found to form chromogens with the estrogens and other steroids which give specific absorption spectra for each compound different from those with concentrated sulfuric acid. The procedure is as follows: Three ml. of reagent grade fuming sulfuric acid (assay: 15–16% free $SO_3$) is added to 30–50 micrograms of steroid in a glass-stoppered test-tube. After one-half hour in the dark at room temperature, the optical density of the solution from 220–600 m$\mu$ is read in a Beckman D. W. spectrophotometer. Quartz cells with ground glass stoppers obtained from Pyrocell Co., New York, were utilized to protect the apparatus from the acid. Fuming sulfuric acid was used as a blank.

Table I summarizes the results obtained with 22 of the steroids studied. The shapes and peaks of the absorption spectra were found to be specific for each compound.

(1) This investigation was supported by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

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(4) A. Zaffaroni and R. Burton, *J. Biol. Chem.*, **193**, 749 (1951).

TABLE I

| Compounds[5] | Absorption maxima |
|---|---|
| Estriol | 430 |
| Estradiol-17$\beta$ | 300, 430 |
| Estradiol-17$\alpha$ | 300, 420 |
| 7-Ketoestrone | 242, 310, 425 |
| Equilenin | 310, 380, 445 |
| Equilin | 305, 380, 435 |
| $\Delta^6$-Dehydroestrone | 300, 365, 435 |
| Estrone | 295, 380 |
| Methoxydoisynolic acid | 265, 320, 390 |
| Diethylstilbestrol | 425 |
| 17$\alpha$-Ethinylestradiol | No maxima |
| 17-Hydroxycorticosterone | 240, 500 |
| 17-Hydroxy-11-dehydrocorticosterone | 295, 440 |
| 17-Hydroxy-11-desoxycorticosterone | 240, 275, 505 |
| Corticosterone | 240, 275, 410, 485 |
| 11-Desoxycorticosterone | 240, 280, 490 |
| Dehydroepiandrosterone | 300, 405 |
| Epiandrosterone | 235, 300, 395 |
| Testosterone | 300 |
| Androsterone | 295, 390 |
| Progesterone | 300, 440 |
| Pregnane-3$\alpha$,20$\alpha$-diol | 285 |

It was furthermore found that the absorption spectra of most compounds change with time so that a new spectrum evolves if the chromogen solution is allowed to stand at room temperature for longer periods of time. For example, 17-hydroxy-11-dehydrocorticosterone after 24 hours exhibits maxima at 250, 280 and 495 m$\mu$. This phenomenon has proven most useful for obtaining the qualitative identification of a single sample of steroid compounds over a period of 24 hours.

(5) Generously donated by Drs. E. Alpert, T. F. Gallagher, E. B. Hershberg, H. B. MacPhillamy, W. H. Pearlman, L. A. Sweat and O. Wintersteiner.

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## A Triazolopyrimidine Analog of 6-Mercaptopurine[1,2]

BY CARL TABB BAHNER, BILL STUMP AND MARY EMMA BROWN

RECEIVED JULY 22, 1953

The compound 6-mercaptopurine has been shown to inhibit growth of certain bacteria[3] and tumors.[4–6] Roblin, Lampen, English, Cole and Vaughn[7] prepared several triazolopyrimidines which were found to inhibit bacterial growth and one of them, 8-azaguanine, was found to inhibit certain tumors.

(1) This research was supported in part by a grant from the Damon Runyon Memorial Fund for Cancer Research and in part by a research grant from the National Institutes of Health, U. S. Public Health Service.

(2) Presented in part at the Southeastern Regional Meeting of the American Chemical Society, Auburn, Alabama, October 24, 1952.

(3) G. B. Elion, G. H. Hitchings and Henry Vanderwerf, *J. Biol. Chem.*, **192**, 505 (1951).

(4) D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock and G. B. Elion, *Proc. Am. Assn. for Cancer Res.*, **1**, 9 (1953).

(5) K. Sugiura, *ibid.*, **1**, 55 (1953).

(6) J. H. Burchenal, D. A. Karnofsky, L. Murphy, R. R. Ellison and C. P. Rhoads, *ibid.*, **1**, 7 (1953).

(7) R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughan, Jr., THIS JOURNAL, **67**, 290 (1945).

A sulfur containing traizolopyrimidine has been reported to inhibit the growth of *Streptococcus faecalis*[8] at very low concentration.

The 8-aza analog of 6-mercaptopurine has been prepared as follows:

Eight grams of 7-hydroxy-1-v-triazolo(d)pyrimidine[9] is added to a solution of 15.0 g. of phosphorus pentasulfide in 233 ml. of boiling pyridine.[10] A clear solution results which begins to deposit crystals as refluxing is continued. After refluxing three to four hours the hot mixture is poured into 400 ml. of boiling water and boiled for a few minutes. Since the product may exist in both oxidized and reduced forms in the same way as cystine–cysteine, care is taken to avoid exposure to atmospheric oxidation. The hot mixture is filtered. The crystals are dissolved in 500 ml. of hot 0.06 *M* KSH solution. The solution is boiled about five minutes, acidified with acetic acid and cooled. The crystals are dissolved in boiling water. The solution is treated with activated charcoal and filtered. The crystals which separate on cooling are dried and treated with hot methanol. The white crystals, 0.9 g. (10%), which separate on chilling the methanol darken about 262° and decompose suddenly about 272°. By paper chromatography using a solvent containing 300 ml. of butanol, 60 ml. of water and 3.6 ml. of glacial acetic acid the $R_f$ is found to be 0.72. The ultraviolet absorption spectrum of a methanol solution shows maxima at 245, 265 and 312 and minima at 235, 260 and 280. Data covering the inhibitory effect of the compound on various tumors are to be published elsewhere. *Anal.* Calcd. for $C_4H_3N_5S$: C, 31.37; H, 1.97. Found[11]: C, 31.60; H, 1.84.

We wish to express our thanks to Dr. Howard Skipper and his associates, of the Southern Research Institute, for their encouragement and for carrying out screening tests. We are indebted to Dr. Gertrude Elion and Dr. George Hitchings of Wellcome Research Laboratories for their interest and for measuring the ultraviolet absorption.

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(9) R. O. Roblin, *et al.*, ref. 8.

(10) *Cf.* E. Klingsberg and D. Papa, THIS JOURNAL, **73**, 4988 (1951).

(11) Analyses by Dr. Harry W. Galbraith, Galbraith Laboratories, Knoxville, Tennessee.

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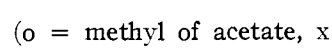
## A Study of the Mechanism of Conversion of Acetate to Cholesterol *via* Squalene[1]

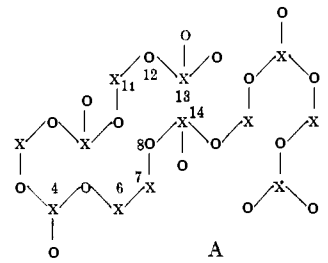
BY WILLIAM G. DAUBEN AND K. H. TAKEMURA

RECEIVED AUGUST 24, 1953

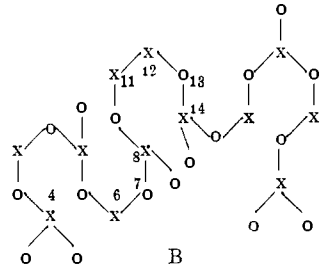
The role of squalene in the biosynthetic mechanism of formation of cholesterol from acetate has received attention in the past year[2,3] and two schemes for its direct cyclization to a steroidal nucleus have been postulated.[4-6] To date, the method employed to evaluate the likelihood of each scheme has been to degrade the labeled cholesterol molecule which has been formed by incubation of labeled acetate with liver slices. Although no degradation experiments have, as yet, been performed with squalene, it has been assumed that the isoprenoid units from which it may have been derived contain 3 methyl groups and 2 carboxyl groups of acetate arranged as shown:



(o = methyl of acetate, x = carboxyl of acetate). The unique character of squalene is its symmetry since it can be viewed as being composed of two sesquiterpenic units linked head-to-head while each sesquiterpenic unit is composed of three isoprenoid units combined in a head-to-tail fashion. Such an arrangement when coupled with the above postulated isotope distribution of the isoprenoid unit requires that the central two carbon atoms of the chain be derived from carboxyl groups of acetate and it is only at this point that two similarly labeled carbon atoms are in a juxtaposition; the remainder of the chain alternates between methyl carbon and carboxyl carbon. With this symmetry feature in mind, the distribution of the labeled atoms in a cholesterol molecule derived by cyclization of squalene following the two previously postulated schemes can be examined.



A



B

It is seen that route A places the two carboxyl carbon atoms under discussion in a juxtaposition at $C_6$ and $C_7$ while in route B it occurs at $C_{11}$ and $C_{12}$. Recently, Cornforth, Hunter and Popjak[7] reported that $C_6$ is derived from a carboxyl carbon atom, as expected in both schemes, and we should like to report the determination of $C_7$.

Cholesterol (I), which had been derived from carboxyl-labeled acetate, was converted into cholesteryl chloride[8] with thionyl chloride and the reduction of the chloride by sodium and amyl alcohol[9] yielded cholest-5-ene (II). Oxidation of II with *t*-butyl chromate[10] gave rise to 7-keto-cholest-5-ene

(1) This work was supported by a grant from the Atomic Energy Commission.

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