

corporation of label into the cytidine moiety only instead of into both cytidine and uridine. Because the skin is unfavourable from both a histological and biochemical point of view, this system cannot be used to resolve the possible mechanism of the effect of DMBA on  $^3\text{H}$ -cytidine incorporation into RNA. Other systems are being investigated with this aim in mind.

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- <sup>1</sup> McCarter, J. A., and Quastler, H., *Nature*, **194**, 873 (1961).  
<sup>2</sup> McCarter, J. A., and Quastler, H., *Biochim. Biophys. Acta*, **55**, 552 (1962).  
<sup>3</sup> Evensen, A., *Acta Pathol. Microbiol. Scand., Supp.*, **148**, 43 (1961).  
<sup>4</sup> Jensen, E. V., Ford, E., and Huggins, C., *Proc. U.S. Nat. Acad. Sci.*, **50**, 454 (1963).  
<sup>5</sup> Elgjo, K., *Nature*, **200**, 608 (1963).  
<sup>6</sup> McCarter, J. A., *J. Nat. Cancer Inst.*, **17**, 399 (1956).  
<sup>7</sup> Feinendegen, L. E., Bond, V. P., and Painter, R. B., *Exp. Cell. Res.*, **22**, 381 (1961).  
<sup>8</sup> Harris, H., *Biochem. J.*, **73**, 362 (1962).  
<sup>9</sup> McMaster-Kaye, R., *J. Histochem. Cytochem.*, **10**, 154 (1962).  
<sup>10</sup> Watts, J. W., and Harris, H., *Biochem. J.*, **72**, 147 (1959).  
<sup>11</sup> Ball, J. K., and McCarter, J. A., *Brit. J. Cancer*, **14**, 577 (1960).  
<sup>12</sup> Harris, H., *Proc. Roy. Soc., B*, **158**, 79 (1963).

### Carrier-free ( $7\text{-}^3\text{H}$ )-Testosterone

DURING the course of clinical investigations being carried out here the need arose for tritium-labelled testosterone of very high specific activity. Although 1,2-tritiated androgens are available<sup>1,2</sup>, their use was precluded in this instance by the extensive loss of radioactivity which would occur during the metabolism of the steroid<sup>3</sup>. Carrier-free ( $7\text{-}^3\text{H}$ )-testosterone acetate was prepared by catalytic reduction in pure tritium of  $17\beta$ -acetoxy-androstan-4,6-dien-3-one.

$\Delta^6$ -Testosterone acetate<sup>4,5</sup>, m.p.  $141.5^\circ\text{--}142.5^\circ$ ,  $\lambda_{\text{max}}^{\text{EtOH}}$  284  $\mu$  ( $\log\epsilon = 4.40$ ),  $\lambda_{\text{max}}^{\text{KBr}}$  5.82 $\mu$ , 6.08 $\mu$ , 6.22 $\mu$ , 6.38 $\mu$ , 8.12 $\mu$ , was prepared by dehydrogenation of testosterone acetate with chloranil in *tert*-butanol<sup>4</sup>. The use of 2,3-dichloro-5,6-dicyano-*p*-benzoquinone catalysed with hydrogen chloride<sup>6</sup> gave the dienone more conveniently and in better yield though mixed with about 10 per cent of the 1,2-unsaturated isomer ( $\lambda_{\text{max}}^{\text{EtOH}}$  244  $\mu$ ). This was unacceptable because chromatographic separation of testosterone acetate and its  $\Delta^1$ - and  $\Delta^6$ -unsaturated derivatives proved very difficult.

The reactivity with respect to catalytic hydrogenation in neutral solution of the steroidal 4,5-double bond is exceeded only marginally by that of the 6,7-double bond<sup>7</sup>. This difference can be increased by conducting the reaction in anhydrous methanol in the presence of a small but critical concentration of alkali<sup>7</sup>. In a preliminary experiment,  $\Delta^6$ -testosterone acetate (42 mg) in dry methanol containing 400 mg l.<sup>-1</sup> of potassium hydroxide was shaken with pre-reduced 5 per cent palladium-on-charcoal catalyst (3.3 mg) under hydrogen at 1 atm. pressure, and allowed to consume 1.2 moles of gas. The solution was filtered, acidified with glacial acetic acid and evaporated. The steroid was separated from inorganic material by leaching with dry chloroform and crystallizing from methanol to yield testosterone acetate, m.p.  $139^\circ\text{--}141^\circ$ ,  $\lambda_{\text{max}}^{\text{EtOH}}$  241  $\mu$  ( $\log\epsilon = 4.21$ ). The mixed melting point with an authentic sample showed no depression. Traces of the  $17\beta$ -acetoxy-androstan-3-ones were detected in the mother-liquor by vapour-phase chromatography.

Using the foregoing conditions, a 60-mg sample of  $\Delta^6$ -testosterone acetate was reduced in carrier-free tritium gas to give testosterone acetate showing no selective absorption at 284  $\mu$  in its ultra-violet spectrum.

This material was saponified with 0.2 N alkali in aqueous methanol and the resulting testosterone separated from saturated steroids by paper chromatography (Whatman No. 1 paper; 19:1 isooctane-toluene/80 per cent aqueous methanol), the zones being detected by autoradiography. The testosterone was eluted from the paper and shown by ultra-violet spectroscopy and carrier-dilution analysis to have a specific activity of 30.8 curies mmole<sup>-1</sup>. This value is consistent with the formulation of the product as ( $7\text{-}^3\text{H}$ )-testosterone, having arisen from the initially formed ( $6,7\text{-}^3\text{H}_2$ )-testosterone by loss of the 6-tritium atom through enolization.

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- <sup>1</sup> Chang, E., and Kenny, N., *Steroids*, **1**, 325 (1963).  
<sup>2</sup> Brodie, H. J., Hayano, M., and Gut, M., *J. Amer. Chem. Soc.*, **84**, 3766 (1962).  
<sup>3</sup> Morato, T., Raab, K., Brodie, H. J., Hayano, M., and Dorfman, R. I., *J. Amer. Chem. Soc.*, **84**, 3764 (1962).  
<sup>4</sup> Schaub, R. E., and Weiss, M. J., *J. Org. Chem.*, **26**, 3915 (1961).  
<sup>5</sup> Djerassi, C., Rosenkranz, G., Romo, J., Kaufmann, S., and Pataki, S., *J. Amer. Chem. Soc.*, **72**, 4534 (1950).  
<sup>6</sup> Caspi, E., Cullen, E., and Grover, P. K., *J. Chem. Soc.*, 212 (1963). Ringold, H. J., and Turner, A., *Chem. and Ind.*, 211 (1962).  
<sup>7</sup> Shepherd, D. A., Donia, R. A., Campbell, J. A., Johnson, B. A., Holysz, R. P., Slomp, G., Stafford, J. E., Pederson, R. L., and Ott, A. G., *J. Amer. Chem. Soc.*, **77**, 1212 (1955).

### Saturated Hydrocarbons from Autoxidizing Methyl Linoleate

EXAMINATION of the vapours of autoxidizing methyl linoleate by gas-liquid chromatography has revealed the presence of several compounds with retention times corresponding to methane, ethane, propane, butane and pentane. Identification of these compounds from autoxidation of a purified unsaturated ester has not, to our knowledge, been previously reported. This finding confirms a prediction made by C. D. Evans *et al.* that low-molecular-weight hydrocarbons would be produced by autoxidation of unsaturated fatty acids<sup>1,2</sup>. They also reported saturated and unsaturated short chain hydrocarbons had been found in products from autoxidation of soybean oil<sup>3</sup>. Buttery and associates identified C<sub>1</sub> to C<sub>5</sub> normal saturated hydrocarbons in dehydrated potatoes, in which linoleic acid is the principal unsaturated acid in the lipids<sup>4</sup>. They speculated that the hydrocarbons might have been formed in autoxidizing dehydrated potatoes by hexanal decomposition that had been catalysed by fat peroxides. However, our findings with methyl linoleate show that the saturated hydrocarbons arise early in the autoxidation process, when aldehydes are either absent or present in undetectable amounts. Therefore, the hydrocarbons are being formed either by an extremely rapid decarboxylation of aldehydes present at a low steady-state concentration or by a totally different mechanism. Japanese workers identified ethane and higher molecular weight alkanes and alkenes from air-oxidation of soybean oil at 240°C (refs. 5 and 6). Their results cannot be compared with ours, however, because our ester was relatively pure and our experiments were performed at room temperature.

We chose methyl linoleate as a simplified system for studying rancidification in parboiled wheat products (bulgur), because linoleic acid is the principal unsaturated fatty acid in the lipids of whole wheat<sup>7</sup>. The original purpose of the experiments was to observe the development of carbonyls produced by autoxidation of unsaturated fatty acids in a system free of the complications inherent in bulgur.