SECTION A, BIOLOGICAL SCIENCES

SUBSECTION MICROBIOLOGY

Method for Identifying Intermediates in the Microbial Utilization of Steroids[1]

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Experiments designed to study the effect of microorganisms on steroids indicate that these compounds may be used as a sole source of carbon and energy for aerobic growth. The organisms involved apparently are cap-

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able of attacking a number of different structural configurations as reported by Durham and Altieri (1958). There are several methods by which the degradation intermediates of these compounds may be identified. One of the most promising is paper chromatography and extensive studies have been conducted to clarify this technique. Neher (1958) has reviewed these procedures and it is interesting to note that the majority of experimentation has been conducted employing filter paper impregnated with various solvents prior to spotting the steroids for development of the chromatogram. These impregnation procedures are time consuming and, in many cases expensive, since the impregnating and blotting is a rather slow process, and the solvents should be redistilled to insure purity. To overcome these disadvantages we have developed a technique that gives rapid and reliable separation of steroid compounds and does not require pretreatment of the paper.

A number of different solvents in various proportions and combinations were studied. We observed that a solvent composed of ethanol-hexane-water-propylene glycol (5:7:15:1) proved to be the most satisfactory for separation of the test compounds. Descending chromatographic techniques were employed in which the compounds were spotted 3 inches from one end of the paper, placed in a paper-lined glass cylinder, equilibrated for 1 hour before adding solvent, and allowed to develop for 14 hours. All experimentation was conducted in a constant temperature room at 25°C. At this time the chromatogram was removed and dried in air for 1 to 2 hours. The steroids were then detected by placing the paper over an ultraviolet light source and covering with a plate glass coated with phosphor.

The steroids and respective Rf values are as follows: progesterone, 0.61; testosterone, 0.89; corticosterone, 0.79; 16 alpha-hydroxyprogesterone, 0.85; and delta 4-androstene-3,17 dione, 0.74. Results from additional experiments indicate that the Rf values for these compounds on untreated paper are somewhat higher than the values obtained on paper impregnated with propylene glycol.

Data obtained from studies comparing this procedure with other methods indicate this technique is fast, gives good separations, and offers definite advantages for separating many of the oxygenated steroid compounds.

LITERATURE CITED

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Neher, R. 1958. Determination of individual adrenocortical steroids. In *Advances in Clinical Chemistry*. Academic Press, New York, Vol. 1: 127-192.