

the mycelium of *P. chrysogenum* was predominantly the L-enantiomorph and is confirmed by the isolation of isopenicillin N from a *P. chrysogenum* fermentation by Flynn *et al.*³. Isopenicillin N and our penicillin M appear to be the same compound and both may well be the same as penicillin W detected by Hale, Miller and Kelly⁹. The presence of δ -aminoadipylpenicillin in *P. chrysogenum* fermentation is of interest in connexion with the pathway for penicillin formation involving the δ -(α -aminoadipyl)-cyst(e)inylvaline tripeptide as suggested by Arnstein and Morris⁸.

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Separation, Purification and Identification of Some Common Barbiturates in Toxicological Analyses

BARBITURATES isolated from biological fluids and tissues are not sufficiently pure for characterization by crystal tests. Purification and separation, if one or more barbiturates are present in the isolated product, are prerequisites for such tests. Paper chromatography and sublimation techniques have been reported in numerous publications to attain sufficient purity of barbiturates for these purposes. Sublimation technique for purification is not consistently successful on barbiturates isolated from biological specimens. A combination of paper chromatography and sublimation was successfully applied to obtain the barbiturates in pure state for characterization by

crystal tests. This method was used by us on micro-quantities of barbiturates isolated from blood and other tissue materials.

Chromatography. Barbiturates were extracted from the biological specimens in the usual manner. The impure extract was spotted on Whatman No. 1 filter paper strips and the chromatography carried out according to the method of Neil and Payton¹. A much better separation was obtained from a mixture of barbital, phenobarbital and butabarbital, when 10 per cent dioxane in methanol was used as the stationary phase. Barbital, phenobarbital, butabarbital, sandoptal, seconal, amobarbital, pentobarbital and cyclopal were purified and separated by this technique. A mixture of amobarbital and pentobarbital could not be separated completely by this technique. When the latter was applied in the form of a band, slight separation occurred and the two barbiturates were obtained in sufficiently pure state by cutting the topmost and bottommost parts of the spot in 2 mm wide strips. Chromatograms were exposed to ammonia and observed under ultra-violet light (253 m μ wave-length), when dark spots of barbiturates were readily indicated.

Sublimation. A modified Eder type of sublimation apparatus (Fig. 1) was used for subliming the barbiturates directly from the paper. The apparatus consisted of cold finger (C), cover-slip (G), attached to the flat end of the cold finger with the help of silicone or high vacuum grease, the outer vessel (B) with bulb (A) and vacuum outlet (D).

The barbiturate spot was cut out from the paper chromatogram and placed in the bulb (A) in the form of thin strips. The cold finger, with cover-slip attached to it, was pushed through the rubber cork as far down as it would go and rest over the opening of the bulb. The bulb was immersed in an oil bath and heated to the desired temperature (80°–100° C). Vacuum was applied through (D) (0.5–1.0 mm mercury pressure). After 4–5 h the cover-slip was removed and crystals observed under the microscope. One tiny drop of 6 N ammonia was placed over a few crystals on the cover-slip. When the crystals were dissolved, one microdrop of concentrated sulphuric acid was added. The characteristic crystals thus produced were observed under the microscope.

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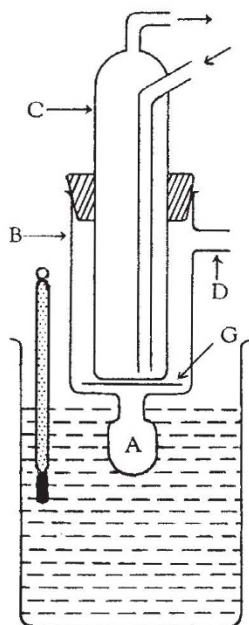


Fig. 1

In vivo Anti-Tumour Activity of Ethyl Heptyloxyacetate against Ehrlich Ascites Cells

PREVIOUS investigations¹⁻⁵ have shown that whole royal jelly, a fraction from royal jelly (10-hydroxy-2-decanoic acid) and certain closely related dicarboxylic acids, some of which are also found in royal jelly⁶, will inhibit the development of transplantable AKR leukaemia and ascites tumours when added *in vitro* just prior to transfer. This activity was associated mainly with 9- and 10-carbon straight-chain monocarboxylic acids, particularly decanoic acid in the ethyl ester form. These compounds showed no activity *in vivo*.

To test the theory that lack of activity *in vivo* might be due to a rapid degradation of the organic acid or its ester due to β -oxidation, 1-¹⁴C-decanoic acid was injected both subcutaneously and intraperitoneally into Swiss mice. Within 3–5 min ¹⁴CO₂ was respired, and 60 per cent of the injected material was respired in the first hour in both instances.

In order to block the β -oxidation, ethyl heptyloxyacetate was synthesized in which an oxygen atom was substituted for the third carbon atom of decanoic acid. When