Determination of lanthionine by thin-layer chromatography

Lanthionine is formed by the breakdown of cystine bonds and in general by the action of chemical reagents such as alkali, or high temperature[1–4]. For this reason the determination of lanthionine is used as a means of detecting the degree of damage to wool. Some methods of determination of lanthionine have been described by different authors and most of them are based on chromatographic techniques[5–7].

During our work on the investigation of the action of ionizing radiations on wool, we have developed a simple method of separation and determination of lanthionine in hydrolysates. This method is based on a thin-layer chromatographic (TLC) separation of lanthionine and, while maintaining the same sensitivity, it is more rapid than the other methods.

The technique we employed was as follows.

200–300 mg of accurately weighed samples of Merino 64's wool were hydrolysed in sealed tubes with 25 ml of 6 *N* HCl for 24 h at 105°. After filtration and evaporation under vacuum to dryness, each hydrolysate was made up to 25 ml with 0.1 *N* HCl. Lanthionine was separated from the other compounds of the hydrolysate by means of ascending chromatography on Cellulose MN 300 G (Macherey and Nagel Co). 20 g of Cellulose MN 300 G and 120 ml of water were shaken vigorously and the slurry was applied to 20 × 20 cm glass plates as a uniform layer 0.25 mm thick. The plates were then allowed to dry 12 h at room temperature.

Portions of 2.0–20.0 μl of wool hydrolysate were then applied to the plates and

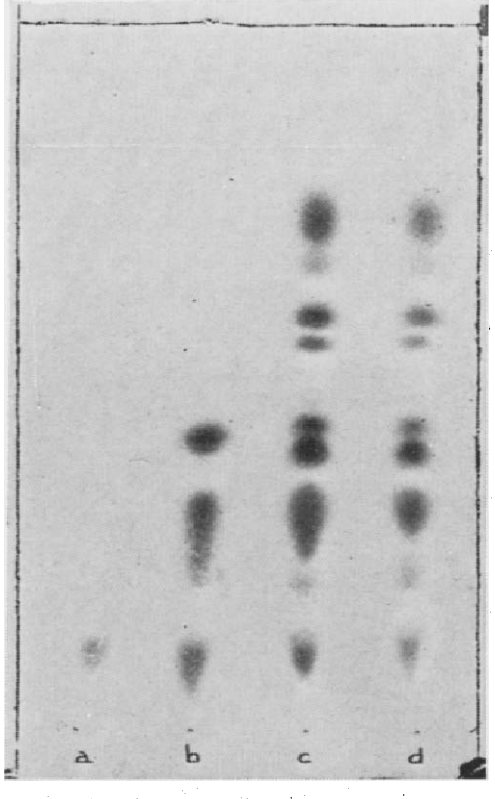

Fig. 1. Thin-layer chromatograms of: (a) lanthionine; (b) reference mixture; (c) untreated Merino 64's wool; (d) alkali treated Merino 64's wool.

the chromatograms were developed with the upper layer of *n*-butanol–acetic acid–water (4:1:5). The plates were dried in a draught of air at 40° for 1 h and the control strips were visualised by spraying with a 1 % solution of ninhydrin in acetone.

Under these conditions lanthionine has an R_F of 0.14 as shown in Fig. 1.

The lanthionine band, located by means of the test strips, is cut out, eluted three times with 1 ml of glacial acetic acid and centrifuged. After centrifugation 1 ml of Chinard's reagent (1.25 ml of ninhydrin dissolved in 20 ml of 6 *N* orthophosphoric acid and 30 ml of glacial acetic acid) was added to the solution and after thorough mixing heated for 10 min in a boiling water bath.

The solution was made up to 5 ml with glacial acetic acid and the lanthionine content was estimated reading the optical density at 450 nm on a Beckman DU spectrophotometer.

The two spots at R_F 0.15 and 0.16 are due to cystine and cysteic acid. As shown in Table I less than 0.25 μmoles of cystine and cysteic acid do not interfere with the determination of lanthionine.

The effect of some factors such as type of tank, plates, spot position, time of separation and spectrophotometric determination of samples were investigated by performing 32 determinations arranged in two 4 × 4 Latin squares[8]. The factors above do not introduce any extra variation into the precision of the method.

The results of some determinations of lanthionine are shown in Table II. The

TABLE I

REACTION OF CHINARD'S REAGENT WITH LANTHIONINE

Amount (μmoles)	*Optical density at 450 mμ in presence of*	
	Cysteic acid	*Cystine*
—	0.095	0.095
0.25	0.095	0.097
0.50	0.088	0.073
1.00	0.069	0.052
2.00	0.047	0.038

TABLE II

PRECISION OF LANTHIONINE DETERMINATIONS

Sample: (A) Standard solution of lanthionine. (B) Untreated Merino 64's wool. (C and D) Alkali treated Merino 64's wool.

Sample	*Amount of lanthionine (μg)*	*S.D. of single determ. (μg)*	*C.V. (%)*
A	10.00	0.53	3.70
B	3.48	0.41	9.60
C	5.78	0.49	6.59
D	5.04	0.37	5.50

standard deviation and the 95 % confidence limits of the single determinations are also reported. The reported data agree with theoretical values.

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Thin-layer and gas chromatography of trimethylsilyl ethers of glycols

Trimethylsilyl derivatives have proved useful in the chromatographic identification of many organic compounds containing replaceable hydrogen atoms[1]. We describe the following procedures which we have employed in identifying a number of cyclic glycols and phenylethylene glycols as metabolic products of unsaturated compounds.

A solution or extract containing the glycol was placed in a glass-stoppered 15-ml conical centrifuge tube and evaporated to dryness. The residue was dissolved in 0.14 ml of pyridine, after which 40 μl of hexamethyldisilazane and 20 μl of trimethylchlorosilane were added, mixing after each addition. The tube was then stoppered and maintained at an appropriate temperature for an appropriate period of time. If an elevated temperature was required, the glass-stoppered top of the tube was wrapped with aluminum foil to prevent seepage of condensate, shaken gently in a thermostatted water bath, and cooled to room temperature before opening. The reaction mixture could be kept overnight at room temperature without loss of sensitivity. Aliquots of the reaction mixture were directly injected in the gas chromatograph, or else the contents of the tube were evaporated to dryness under a stream of dry nitrogen passed through a capillary tube extending to just above the surface of the liquid. The residue was leached with 0.5 ml of hexane, and aliquots of the extract used for thin-layer or gas chromatography. If it was desired to concentrate the extract, evaporation was performed under nitrogen.

For thin-layer chromatography, Eastman Chromagram sheets No. 6060 (coated with silica gel containing a fluorescent indicator) were activated at 110° for 15 min. Spots were applied without using an air stream for drying. Ascending chromatography was carried out with heptane. The sheets were then dried at room temperature and the spots located under U.V. (2537 Å) light.