phyllized leaf material was wetted with ethanol and hydrolysed with 0·33 N potassium hydroxide for periods of 1, 2, 3, 4, 5, 6 and 18 h. Samples in duplicate were passed over columns of '*AG* 1-8*X*' 100–200 mesh chloride form of 'Dowex'; the RNA was determined by absorption at 260 mμ. Yeast RNA purified by similar treatment was used as the standard. Results are given in Table 1.

Table. 1. Effect of Exposure Time on Hydrolysis of Leaf Tissue RNA (0·33 N Potassium Hydroxide at 37° C)

Sample (*A* and *B*)	Exposure time (h)	RNA μg/g fresh weight	Percentage of recovery
1	1	2,140	62
2	2	2,520	73
3	3	2,550	74
4	4	2,720	79
5	5	2,900	84
6	6	3,330	96
7	18	3,450	100

These results indicated that more than 60 per cent of the RNA became acid-soluble nucleotides after 1 h exposure but this material required at least 6 h exposure for complete hydrolysis, based on 18 h as 100 per cent. Since 6 h exposure failed to completely solubilize the RNA, hydrolysis time was lengthened to 8 h as shown in Table 2.

Table 2. Effect of Exposure Time on Hydrolysis of Leaf Tissue RNA (0·33 N Potassium Hydroxide at 37° C).

Sample (*A* and *B*)	Exposure time (h)	RNA μg/g fresh weight	Percentage of recovery
8	6	3,350	97
9	7	3,600	104·4
10	8	3,500	101·6
11	18	3,440	100

Extension of hydrolysis beyond 7 h appears to be unnecessary. Seven-hour hydrolysis actually gave a somewhat higher reading than the standard 18 h. When repeated in triplicate at 7 and 18 h, the former time gave 101·4 per cent RNA content, relative to the latter.

These investigations indicate that hydrolysis of treated leaf tissue with 0·33 N potassium hydroxide for periods beyond 7 h will not yield additional RNA in the form of acid-soluble nucleotides. However, unlike animal tissues, lesser periods of hydrolysis are insufficient for complete hydrolysis.

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Amino-acid Configuration of Mycobacillin

Mycobacillin, a new antifungal antibiotic isolated from the culture filtrate of a strain of *Bacillus subtilis*, is a cyclic peptide containing 13 residues of 7 different amino-acids, the sequence of which has also been worked out[1-2]. Now the stereotypic nature of the constituent amino-acids is an interesting query. Antibiotics are often characterized by the presence of D-amino-acids. Penicillin and antibiotics of actinomycin group contain D-valine[3]. Actinomycin also contains D-alloisoleucine. Likewise, D-phenylalanine is the constituent of gramicidine *S*[4] and tyrocidines[5]. Extreme examples are, however, polymyxin-*B*, polymyxin-*D* and bacitracin *A*. Polymyxins contain D-phenylalanine, D-leucine, D-serine and D-α.γ-diaminobutyric acid, and bacitracin contains D-ornithine, D-glutamic acid, DL-aspartic acid and D-phenylalanine[6]. This communication records the stereotypic nature of each of the constituent amino-acids of mycobacillin. In essence the method is based on the substrate specificity of D- and L-amino-acid oxidases and L-glutamic acid dehydrogenase.

Mycobacillin isolated in state of chemical purity was subjected to complete acid hydrolysis. The acid was removed and acid-free hydrolysate was dissolved in water. The amino-acid mixture so obtained was incubated with hog kidney D-amino-acid oxidase[7] and snake venom L-amino-acid oxidase[8] separately. Another aliquot of the mixture was treated with ox-liver L-glutamic acid dehydrogenase[9]. The amino-acid mixture after incubation was chromatographed to determine if any disappearance of amino-acids has occurred. The spots when obtained were quantitatively assayed.

It appears from Table 1 that leucine is oxidized completely and tyrosine almost completely by L-amino-acid oxidase while they are left practically untouched by D-amino-acid oxidase. L-Configuration of these two amino-acids are thus established. Now L-amino-acid oxidase does not act on L-alanine, L-serine, L-proline and L-aspartic acid[12], which were found to be equally inactive towards D-amino-acid oxidase tested; hence they are of L- variety. Regarding aspartic acid, it may be pointed out that this amino-acid has been repeated as many as five times in the molecule[2] and that about four-fifths of it was oxidizable by D-amino-acid oxidase. This may be taken to mean that any four out of five aspartic acid residues are of D-isomer. Now for glutamic acid, neither D- nor L- is found to be acted on by either of the oxidases. It is similarly recovered when treated with L- specific crystalline ox-liver glutamic acid dehydrogenase. So glutamic acids present in the molecule are of D-configuration.

Table 1. Oxidation of Constituent Amino-Acids by Amino-Acid Oxidases

Amino-acid	Per cent disappeared after D-amino-acid oxidase treatment	Per cent disappeared after L-amino-acid oxidase treatment
Alanine	nil	nil
Leucine	nil	96·0
Tyrosine	18·0	89·0
Serine	6·6	nil
Proline	4·0	nil
Aspartic acid	76·0	nil
Glutamic acid	nil	nil

Incubation mixture consists of pyrophosphate buffer *p*H 8·3 (0·1 M), 1·00 ml., FAD, 0·01 μM, catalase 100 units, neutralized mycobacillin hydrolysate and either D- (5 mg) or L- (1 mg) amino-acid oxidase. The reaction was carried out at 37° and after 180 min was terminated by addition of double the volume of ethanol. Amino-acids were then purified on 'Dowex-50 column'[10] and fractionated by two-dimensional paper chromatography using *n*-butanol : acetic acid : water (4 : 1 : 1) and water-saturated phenol as developing solvents. The amino-acids were estimated by the method of Giri *et al.*[11]. The estimation of amino-acids in mycobacillin hydrolysate was done similarly after fractionation and it was taken as control.

The stereotypic configuration of the constituent amino-acids of mycobacillin are thus indicated.

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