of water R and 9 volumes of sulphuric acid R. Heat on a water-bath for 3 min. Cool in ice water and add 5 ml of acetic anhydride R. No violet-red colour develops (0.05 per cent).

Chlorides (2.4.4). Dissolve 1.0 g with heating in 8 ml of acetic acid R, cool and dilute to 10 ml with the same acid. Dilute 5 ml of the solution to 15 ml with water R. The solution complies with the limit test for chlorides (100 ppm).

Loss on drying (2.2.32). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 80 °C (bp) at a pressure not exceeding 0.7 kPa for 4 h.

Sulphated ash (2.2.14). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 50 ml of anhydrous acetic acid R. Add 0.2 ml of naphtholbenzenz solution R and titrate with 0.1 M perchloric acid until the colour changes from orange to green.

1 ml of 0.1 M perchloric acid is equivalent to 28.44 mg of Cr₂H₂N₂O₂.

STORAGE
Store protected from light.

IMPURITIES

A. N(pyrin-4-ylmethyl)ethanamine,

B. N-ethyl-2-phenyl-N(pyrin-4-ylmethyl)propenamide,

C. (2RS)-3-hydroxy-2-phenylpropanoic acid (tropic acid).

ASSAY
Dissolve 0.100 g in carbon dioxide-free water R and dilute to 10.0 ml with the same solvent.

Appearance of solution. Solution S is not more opalescent than reference suspension III (2.2.1).

pH (2.2.3). The pH of solution S is 3.0 to 6.0.

Absorbance (2.2.25). Dissolve 30.0 mg in 0.001 M hydrochloric acid and dilute to 100 ml with the same acid. The solution shows an absorption maximum at 280 nm and a minimum at 250 nm. The specific absorbance at the maximum is 13.5 to 16.5 and that at the minimum is not greater than 7.0.

Chymotrypsin. To 1.8 ml of buffer solution pH 8.0 R add 7.4 ml of water R and 0.5 ml of 0.2 M acetyltoxysine ethyl ester R. While shaking the solution, add 0.3 ml of solution S and start a stop-watch. After exactly 5 min, measure the pH (2.2.3) (test solution). Prepare a reference solution in the same manner, replacing solution S by 0.3 ml of a 0.5 g/l solution of chymotrypsin BRP and measure the pH (2.2.3) exactly 5 min after adding the chymotrypsin. The pH of the test solution is higher than that of the reference solution.

Loss on drying (2.2.32). Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C at a pressure not exceeding 670 Pa for 2 h.

Microbial contamination. Total viable aerobic count (2.6.12) not more than 10⁵ micro-organisms per gram, determined by plate count. It complies with the tests for Escherichia coli and Salmonella (2.6.15).

ASSAY
The activity of trypsin is determined by comparing the rate at which it hydrolyses benzoylarginine ethyl ester hydrochloride R with the rate at which trypsin BRP hydrolyses the same substrate in the same conditions.

Apparatus. Use a reaction vessel of about 30 ml capacity provided with:
– a device that will maintain a temperature of 25.0 ± 0.1 °C,
– a stirring device (for example, a magnetic stirrer),
– a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration device may be used. For the latter, the burette is graduated in 0.005 ml and the pH meter is provided with a wide-range scale and glass-calomel electrodes.

Test solution. Dissolve sufficient of the substance to be examined in 0.001 M hydrochloric acid and dilute to 25.0 ml with the same acid in order to obtain a solution containing approximately 700 nanokatals per millilitre.

Reference solution. Dissolve 25.0 mg of trypsin BRP in 0.001 M hydrochloric acid and dilute to 25.0 ml with the same acid.

Store the solutions at 0 °C to 5 °C. Warm 1 ml of each solution to about 25 °C over 15 min and use 50 µl of each solution for each titration. Carry out the titration in an atmosphere of nitrogen. Transfer 10.0 ml of 0.0015 M borate buffer solution pH 8.0 R to the reaction vessel and, while stirring, add 1.0 ml of a freshly prepared 6.86 g/l solution of benzoylarginine ethyl ester hydrochloride R. When the temperature is steady at 25.0 ± 0.1 °C (after about 5 min) adjust the pH to exactly 8.0 with 0.1 M sodium hydroxide. Add 50 µl of the test solution and start a timer. Maintain the pH at 8.0 by the addition of 0.1 M sodium hydroxide used per second. Carry out a titration in the same manner using the reference solution and calculate the volume of 0.1 M sodium hydroxide used per second.

Calculate the activity in microkatal per milligram using the expression:

\[
\frac{m'}{m} \times \frac{V}{V'} \times A
\]

\(m\) = mass of the substance to be examined, in milligrams,
\(m'\) = mass of trypsin BRP, in milligrams,
\(V\) = volume of 0.1 M sodium hydroxide used per second by the test solution,
\(V'\) = volume of 0.1 M sodium hydroxide used per second by the reference solution,
\(A\) = activity of trypsin BRP, in microkatal per milligram.

IDENTIFICATION

First identification: A, B.
Second identification: A, C, D.

A. It complies with the test for specific optical rotation (see Tests).
B. Examine the substance by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with tryptophan CRS. Examine the substances prepared as discs.
C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).
D. Dissolve about 20 mg in 10 ml of water R. Add 5 ml of dimethylaminobenzaldehyde solution R6 and 2 ml of hydrochloric acid R1. Heat on a water-bath. A purple-blue colour develops.

TESTS

Appearance of solution. Dissolve 0.1 g in 1 M hydrochloric acid and dilute to 10 ml with the same acid. The solution is clear (2.2.1) and not more intensely coloured than reference solution BY (2.2.2, Method II).

Specific optical rotation (2.2.7). Dissolve 0.25 g in water R, heating on a water-bath if necessary, and dilute to 25.0 ml with the same solvent. The specific optical rotation is ϑ 33.0 to −33.0, calculated with reference to the dried substance.

Ninhydrin-positive substances. Examine by thin-layer chromatography (2.2.27), using a TLC silica gel plate R. Test solution (a). Dissolve 0.10 g of the substance to be examined in a mixture of equal volumes of glacial acetic acid R and water R and dilute to 10 ml with the same mixture of solvents. Test solution (b). Dilute 1 ml of test solution (a) to 50 ml with a mixture of equal volumes of glacial acetic acid R and water R.

Reference solution (a). Dissolve 10 mg of tryptophan CRS in a mixture of equal volumes of glacial acetic acid R and water R and dilute to 50 ml with the same mixture of solvents.

CHARACTERS

A white or almost white, crystalline or amorphous powder, sparingly soluble in water, slightly soluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

STORAGE

Store in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

DEFINITION

Tryptophan contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (S)-2-amino-3-(1H-indol-3-yl)propanoic acid, calculated with reference to the dried substance.