fluoresceinate R. Dry the plate in a current of air and expose to ammonia vapour for a few seconds. Examine in ultraviolet light at 254 nm and at 365 nm. Any band corresponding to nitrosotriaminopyrimidine in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution. Dissolve 0.1 g of the substance to be examined in 20 ml of *dimethyl sulphoxide R*. Dilute 2 ml of the solution to 50 ml with *methanol R*.

Reference solution. Dilute 1 ml of the test solution to 200 ml with *methanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R1*, 10 volumes of *methanol R* and 90 volumes of *ethyl acetate R*. Allow the plate to dry in air until the solvents have evaporated and examine in ultraviolet light at 365 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 5 ml of *anhydrous formic acid R* and add 100 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 25.33 mg of $C_{12}H_{11}N_7$.

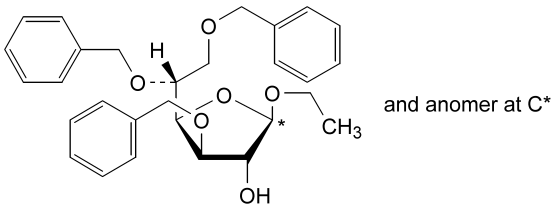
STORAGE

Store protected from light.

01/2008:1740

TRIBENOSIDE

Tribenosidum



$C_{29}H_{34}O_6$ M_r 478.6
[10310-32-4]

DEFINITION

Mixture of α- and β-anomers of ethyl 3,5,6-tri-*O*-benzyl-D-glucofuranoside.

Content: 96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance: yellowish to pale yellow, clear, viscous liquid.

Solubility: practically insoluble in water, very soluble in acetone, in methanol and in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *tribenoside CRS*.

TESTS

Solution S. Dissolve 4.00 g in *methanol R* and dilute to 20 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 420 nm has a maximum of 0.10.

Specific optical rotation (*2.2.7*): – 31.0 to – 40.0.

Dilute 2.0 ml of solution S to 20.0 ml with *methanol R*.

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 1.000 g of the substance to be examined in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 25.0 ml with the same mixture of solvents.

Test solution (b). Dissolve 50.0 mg of the substance to be examined in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (a). Dilute 25.0 mg of *benzaldehyde R* and 30.0 mg of *tribenoside impurity A CRS* to 100.0 ml with *acetonitrile R*. Introduce 20.0 ml of this solution into a 50 ml volumetric flask, add 2.5 ml of *water R* and dilute to 50.0 ml with *acetonitrile R*.

Reference solution (b). Dissolve 50.0 mg of *tribenoside CRS* in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (c). Dissolve 12.0 mg of *benzyl ether R* in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 100.0 ml with the same mixture of solvents.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

– *mobile phase A*: 0.1 per cent *V/V* solution of *phosphoric acid R*,
– *mobile phase B*: *acetonitrile R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	55 → 10	45 → 90
40 - 55	10	90
55 - 56	10 → 55	90 → 45
56 - 60	55	45

Flow rate: 1.3 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl; inject test solution (a) and reference solutions (a), (b) and (c).

Relative retentions with reference to the β-anomer of tribenoside (retention time = about 18 min): α-anomer = about 1.1; impurity C = about 0.2; impurity B = about 0.6; impurity D = about 0.8; impurity A = about 1.4.

System suitability: reference solution (b):

– *resolution*: minimum 3.0 between the peaks due to the α-anomer and to the β-anomer of tribenoside.

Limits:

- *impurity A*: not more than 1.7 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- *impurity C*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent); if the area of the peak due to impurity C in the chromatogram obtained with the test solution is greater than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.25 per cent), dilute the test solution to obtain an area equal to or smaller than the area of the peak in the chromatogram obtained with reference solution (a); calculate the content of impurity C taking into account the dilution factor;
- *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent),
- *any other impurity*: not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.3 per cent),
- *total*: not more than 6.7 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (2.0 per cent),
- *disregard limit*: 0.17 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (*2.4.8*): maximum 20 ppm.

Dilute 5.0 ml of solution S to 20.0 ml with *methanol R*. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with *methanol R*.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

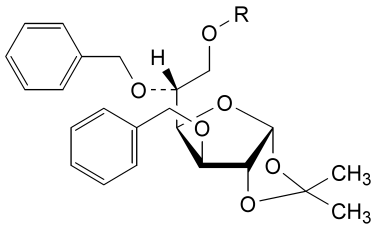
Injection: test solution (b) and reference solution (b).

Calculate the sum of the percentage contents of the α-anomer and the β-anomer of tribenoside.

STORAGE

Under nitrogen, in an airtight container.

IMPURITIES



A. R = CH_2-C_6H_5: 3,5,6-tri-*O*-benzyl-1,2-*O*-(1-methylethylidene)-α-D-glucofuranose,

B. R = H: 3,5-di-*O*-benzyl-1,2-*O*-(1-methylethylidene)-α-D-glucofuranose,

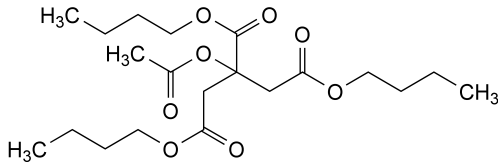
C. C_6H_5-CHO: benzaldehyde,

D. C_6H_5-CH_2-O-CH_2-C_6H_5: dibenzyl ether.

01/2008:1770

TRIBUTYL ACETYLCITRATE

Tributylis acetylcitras



$C_{20}H_{34}O_8$ M_r 402.5

[77-90-7]

DEFINITION

Tributyl 2-(acetyloxy)propane-1,2,3-tricarboxylate.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: clear, oily liquid.

Solubility: not miscible with water, miscible with alcohol and with methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of tributyl acetylcitrate.*

TESTS

Appearance. The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity. Dilute 10 g with 10 ml of previously neutralised *alcohol R*, add 0.5 ml of *bromothymol blue solution R2*. Not more than 0.3 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Refractive index (*2.2.6*): 1.442 to 1.445.

Related substances. Gas chromatography (*2.2.28*).

Test solution. Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Reference solution (a). Dissolve 50 mg of the substance to be examined and 50 mg of *tributyl citrate R* in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution to 20.0 ml with *methylene chloride R*. Dilute 1.0 ml of this solution to 25.0 ml with *methylene chloride R*.

Column:

- *material*: fused silica,
- *size*: *l* = 30 m, Ø = 0.53 mm,
- *stationary phase*: *poly[(cyanopropyl)(methyl)][(phenyl)(methyl)]siloxane R* (film thickness 1.0 μm).

Carrier gas: *helium for chromatography R*.

Linear velocity: 36 cm/s.

Split ratio: 1:20.

Temperature:

- *column*: 200 °C,
- *injection port and detector*: 250 °C.

Detection: flame ionisation.

Injection: 1 μl.

Run time: twice the retention time of tributyl acetylcitrate.