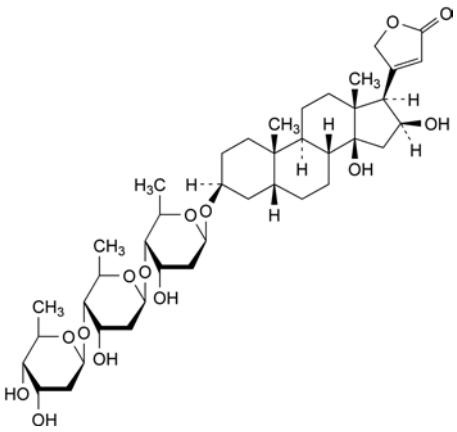
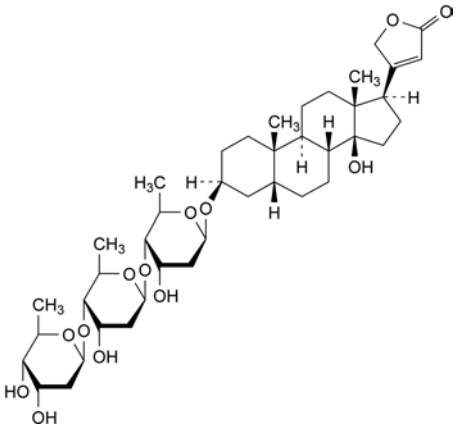
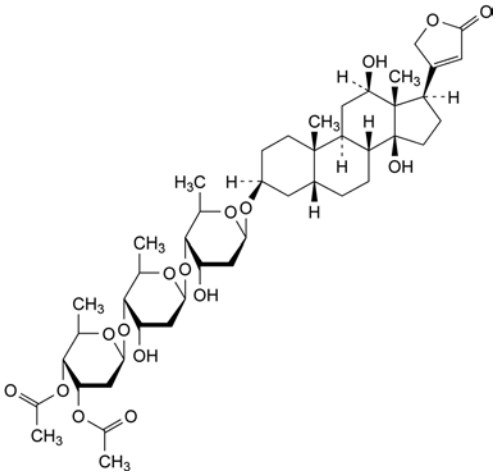


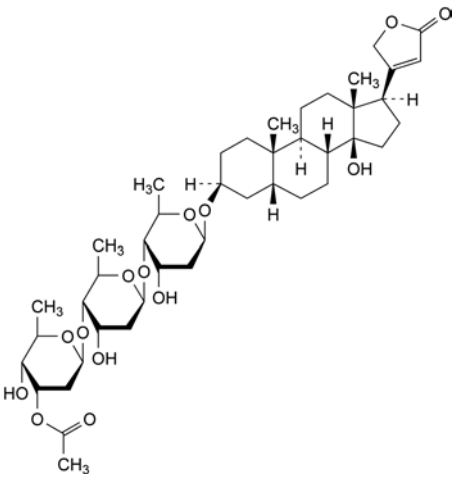
D. 3β-[(2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14,16β-dihydroxy-5β-card-20(22)-enolide (gitoxin),



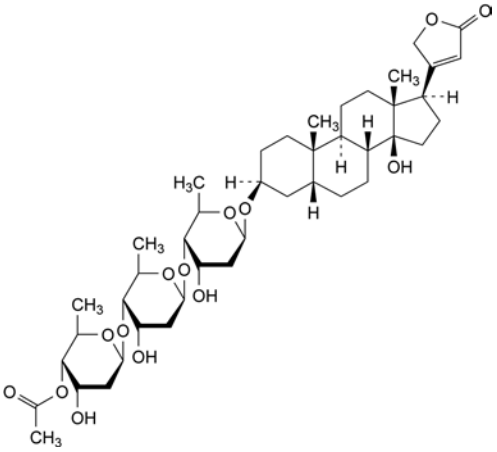
E. 3β-[(2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxy-5β-card-20(22)-enolide (digitoxin),



F. 3β-[(3,4-*O*-diacetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (diacetyldigoxin),



G. 3β-[(3-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxy-5β-card-20(22)-enolide (α-acetyldigitoxin),

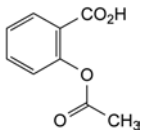


H. 3β-[(4-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxy-5β-card-20(22)-enolide (β-acetyldigitoxin).

01/2011:0309

ACETYLSALICYLIC ACID

Acidum acetylsalicylicum



$C_9H_8O_4$ M_r 180.2
[50-78-2]

DEFINITION

2-(Acetyloxy)benzoic acid.

Content: 99.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: slightly soluble in water, freely soluble in ethanol (96 per cent).

mp: about 143 °C (instantaneous method).

IDENTIFICATION

First identification: A, B.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *acetylsalicylic acid CRS.*

B. To 0.2 g add 4 mL of *dilute sodium hydroxide solution R* and boil for 3 min. Cool and add 5 mL of *dilute sulfuric acid R*. A crystalline precipitate is formed. Filter, wash the precipitate and dry at 100-105 °C. The melting point (*2.2.14*) is 156 °C to 161 °C.

C. In a test tube mix 0.1 g with 0.5 g of *calcium hydroxide R*. Heat the mixture and expose to the fumes produced a piece of filter paper impregnated with 0.05 mL of *nitrobenzaldehyde solution R*. A greenish-blue or greenish-yellow colour develops on the paper. Moisten the paper with *dilute hydrochloric acid R*. The colour becomes blue.

D. Dissolve with heating about 20 mg of the precipitate obtained in identification test B in 10 mL of *water R* and cool. The solution gives reaction (a) of salicylates (*2.3.1*).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1.0 g in 9 mL of *ethanol (96 per cent) R*.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 0.100 g of the substance to be examined in *acetonitrile for chromatography R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 50.0 mg of *salicylic acid R* (impurity C) in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b). Dissolve 10 mg of *salicylic acid R* (impurity C) in the mobile phase and dilute to 10.0 mL with the mobile phase. To 1.0 mL of this solution add 0.2 mL of the test solution and dilute to 100.0 mL with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *phosphoric acid R, acetonitrile for chromatography R, water R* (2:400:600 *V/V/V*).

Flow rate: 1 mL/min.

Detection: spectrophotometer at 237 nm.

Injection: 10 µL.

Run time: 7 times the retention time of acetylsalicylic acid.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.

Relative retention with reference to acetylsalicylic acid (retention time = about 5 min): impurity A = about 0.7; impurity B = about 0.8; impurity C = about 1.3; impurity D = about 2.3; impurity E = about 3.2; impurity F = about 6.0.

System suitability: reference solution (b):

- *resolution*: minimum 6.0 between the peaks due to acetylsalicylic acid and impurity C.

Limits:

- *impurities A, B, C, D, E, F*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

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

Dissolve 1.0 g in 12 mL of *acetone R* and dilute to 20 mL with *water R*. 12 mL of the solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 6 volumes of *water R* and 9 volumes of *acetone R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo*.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In a flask with a ground-glass stopper, dissolve 1.000 g in 10 mL of *ethanol (96 per cent) R*. Add 50.0 mL of *0.5 M sodium hydroxide*. Close the flask and allow to stand for 1 h. Using 0.2 mL of *phenolphthalein solution R* as indicator, titrate with *0.5 M hydrochloric acid*. Carry out a blank titration.

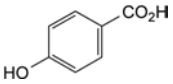
1 mL of *0.5 M sodium hydroxide* is equivalent to 45.04 mg of $C_9H_8O_4$.

STORAGE

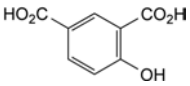
In an airtight container.

IMPURITIES

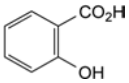
Specified impurities: A, B, C, D, E, F.



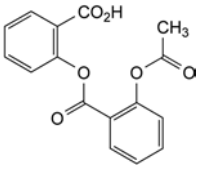
A. 4-hydroxybenzoic acid,



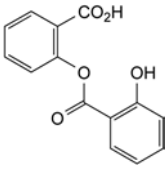
B. 4-hydroxybenzene-1,3-dicarboxylic acid (4-hydroxyisophthalic acid),



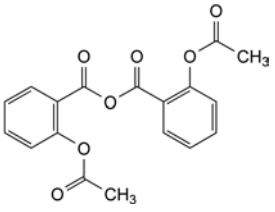
C. 2-hydroxybenzenecarboxylic acid (salicylic acid),



D. 2-[[2-(acetyloxy)benzoyl]oxy]benzoic acid (acetylsalicyl-salicylic acid),



E. 2-[(2-hydroxybenzoyl)oxy]benzoic acid (salicylsalicylic acid),

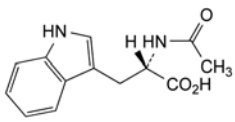


F. 2-(acetyloxy)benzoic anhydride (acetylsalicylic anhydride).

01/2009:1383
corrected 7.0

N-ACETYLTRYPTOPHAN

N-Acetyltryptophanum



and enantiomer

$C_{13}H_{14}N_2O_3$ M_r 246.3
[87-32-1]

DEFINITION

(*RS*)-2-Acetylamino-3-(1*H*-indol-3-yl)propanoic acid.

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

PRODUCTION

Tryptophan used for the production of *N*-acetyltryptophan complies with the test for impurity A and other related substances in the monograph on *Tryptophan (1272)*.

CHARACTERS

Appearance: white or almost white, crystalline powder, or colourless crystals.

Solubility: slightly soluble in water, very soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

mp: about 205 °C.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D, E.

A. Optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *N-acetyltryptophan CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 50 mg of the substance to be examined in 0.2 mL of *concentrated ammonia R* and dilute to 10 mL with *water R*.

Reference solution (a). Dissolve 50 mg of *N-acetyltryptophan CRS* in 0.2 mL of *concentrated ammonia R* and dilute to 10 mL with *water R*.

Reference solution (b). Dissolve 10 mg of *tryptophan R* in the test solution and dilute to 2 mL with the test solution.

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: *glacial acetic acid R, water R, butanol R* (25:25:40 *V/V/V*).

Application: 2 µL.

Development: over a path of 10 cm.

Drying: in an oven at 100-105 °C for 15 min.

Detection: examine in ultraviolet light at 254 nm.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 2 mg in 2 mL of *water R*. Add 2 mL of *dimethylaminobenzaldehyde solution R6*. Heat on a water-bath. A blue or greenish-blue colour develops.

E. It gives the reaction of acetyl (*2.3.1*). Proceed as described for substances hydrolysable only with difficulty.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 or GY_7 (*2.2.2, Method II*).

Dissolve 1.0 g in a 40 g/L solution of *sodium hydroxide R* and dilute to 100 mL with the same alkaline solution.

Optical rotation (*2.2.7*): – 0.1° to + 0.1°.

Dissolve 2.50 g in a 40 g/L solution of *sodium hydroxide R* and dilute to 25.0 mL with the same alkaline solution.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the test and reference solutions immediately before use.*

Buffer solution pH 2.3. Dissolve 3.90 g of *sodium dihydrogen phosphate R* in 1000 mL of *water R*. Add about 700 mL of a 2.9 g/L solution of *phosphoric acid R* and adjust to pH 2.3 with the same acid solution.

Solvent mixture: *acetonitrile R, water R* (10:90 *V/V*).

Test solution. Dissolve 0.10 g of the substance to be examined in a mixture of 50 volumes of *acetonitrile R* and 50 volumes of *water R* and dilute to 20.0 mL with the same mixture of solvents.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (b). Dilute 4.0 mL of reference solution (a) to 100.0 mL with the solvent mixture.

Reference solution (c). Dissolve the contents of a vial of *1,1′-ethylidenebistryptophan CRS* in 1 mL of reference solution (b).

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 40 °C.

Mobile phase:

– *mobile phase A*: *acetonitrile R*, buffer solution pH 2.3 (115:885 *V/V*);
– *mobile phase B*: *acetonitrile R*, buffer solution pH 2.3 (350:650 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 45	100 → 0	0 → 100
45 - 65	0	100

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 µL of the test solution and reference solutions (a) and (c).

Retention time: *N*-acetyltryptophan = about 29 min; 1,1′-ethylidenebis(tryptophan) = about 34 min.

System suitability: reference solution (c):

– *resolution*: minimum 8.0 between the peaks due to *N*-acetyltryptophan and 1,1′-ethylidenebis(tryptophan); if necessary, adjust the time programme for the elution gradient (an increase in the duration of elution with mobile phase A produces longer retention times and a better resolution);
– *symmetry factor*: maximum 3.5 for the peak due to 1,1′-ethylidenebistryptophan in the chromatogram obtained with reference solution (c).

Limits:

– *impurities A, B, C, D, E, F, G, H, I, J, K, L*: for each impurity, not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);