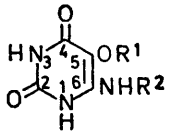
Synthesis of Convicine (6-Amino-5-β-D-glucopyranosyloxyuracil)

By **Shlomo Bien,* Drorah Amith,** and (in part) **Menuha Ber,** Department of Chemistry, Technion–Israel Institute of Technology, Haifa, Israel

Glycosidation of 6-acetamido-5-hydroxyuracil (1c) with tetra-*O*-acetyl-β-D-glucopyranosyl bromide gave the penta-acetate (1d), identical with the penta-acetate of natural convicine. Deacetylation of (1d) yielded the free glycoside, identical with convicine itself. In an alternative approach 1-benzylconvicine penta-acetate (8) was synthesised by a multi-step sequence from 5-tetrahydropyranyloxyuracil (2). Attempted debenzylation of compound (8) failed.

CONVICINE (1a) was discovered by Ritthausen [1] in *Vicia sativa* L. and later also found [2,3] in *Vicia faba* L. (fava beans). It was identified as a β-glucoside of 6-amino-5-hydroxyuracil (1b) by Johnson, who suggested that the glucose was attached to the aglycone at N-1.[3,4] Much later, Bendich and Clements [5] suggested that the glucose unit was attached to oxygen at C-5. This has recently been confirmed by degradative and spectroscopic evidence.[6] The biological importance [7,8] of convicine stimulated us to attempt its synthesis.

The reagent of choice for phenol glycosidation is tetra-*O*-acetyl-β-D-glucopyranosyl bromide,[9,10] in the presence of alkali as acid acceptor. In applying this to 6-amino-5-hydroxyuracil, competing nucleophilic attack on the bromo-sugar by the 6-NH_2 group was avoided by use of the *N*-acetyl derivative (1c). The lack of adequate solubility of this compound in dry aprotic solvents explains the failure of glycosidation when silver oxide or carbonate is used as acid acceptor in the classical Koenigs–Knorr condensation.[9] The procedure of Helferich,[11] with alkaline hydroxide in aqueous acetone,



(1)

	R^1	R^2
a;	Glu	H
b;	H	H
c;	H	Ac
d;	$(OAc)_4$Glu	Ac

Glu = β-D-glucopyranosyl

was found more promising, and the penta-acetate (1d) was successfully synthesised by applying the Hibbert modification: [12] substitution of the potassium for the sodium salt of the aglycone, operating at 0° throughout the reaction, and carefully controlling the pH of the medium during addition of the reagents.

Even under these strictly controlled conditions the yield of the condensation was low (*ca.* 10%), and uncoupled sugar was detected as glucose tetra-acetate.† This may indicate a greater rate for the solvolytic decomposition of the glycosyl bromide as compared with the product-forming nucleophilic attack by the aglycone anion in the aqueous medium. Another possible explanation might involve competing anion formation from one of the NH groups (see later).

Acylaminopyrimidines are reported to need vigorous hydrolysis to give the parent amine.[13] In contrast, the 6-acetamido-group was found to undergo cleavage under mild conditions and in one step together with the acetoxy-groups of the sugar system.‡ Thus treatment of (1d) with methanolic sodium methoxide at room temperature gave the free glycoside, identical with natural convicine (m.p., mixed m.p.; i.r. and n.m.r. spectra).

We hoped to increase the yield of the coupling by use of the easily available 1-benzyl derivative (7) as aglycone. The protection was thought necessary to avoid dissociation of the lactam proton, a process competing with anion formation at the C(5)–OH in aqueous alkaline medium. Benzylconvicine penta-acetate (8) was in fact obtained in 40% yield. However the fact that by this approach the yield was raised is not conclusive evidence that the suggested competing anion formation is the only reason for the low-yield condensation of (1c).§

All efforts to effect hydrogenolytic debenzylation of (8) were fruitless; starting material only was recovered. Similar difficulties were reported by Japanese workers [14] who found 3-benzyluridine to be resistant to prolonged catalytic hydrogenation.¶

The aglycone (7) was synthesised by a number of steps (see Scheme) from the tetrahydropyranyl ether

† Unfortunately no by-products derived from the pyrimidine system were isolated, presumably owing to the instability of the molecule (1b) in alkaline medium.[8]

‡ The cleavage is apparently facilitated by the reduction in basicity of the C-6 nitrogen atom by attachment to an enone system.

§ Although glycosidation of the corresponding 1,3-dibenzyl derivative (thus avoiding possible anion formation at both N-1 and N-3) may have put some light on this question, no investigation was undertaken in this direction.

¶ We thank a referee for drawing our attention to this work.

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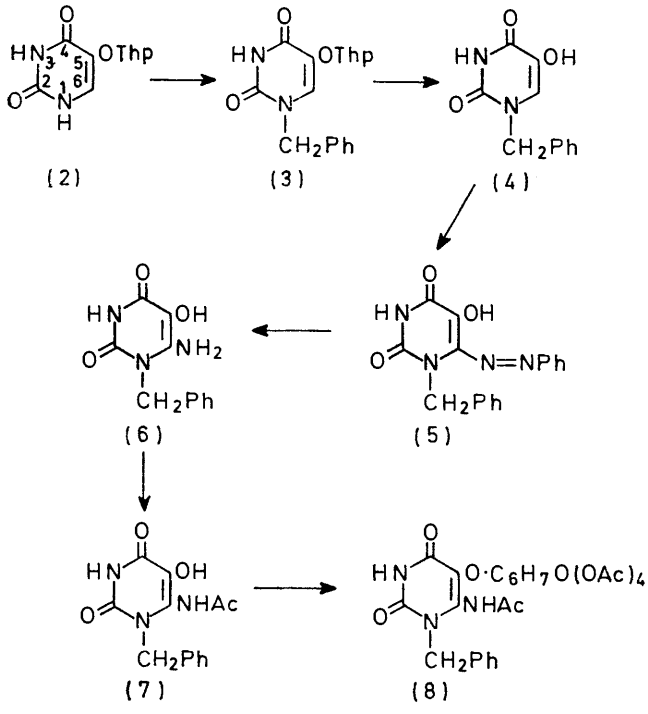
(2), which in turn was prepared from ethyl tetrahydropyran-2-yloxyacetate.[15] Monobenzylation of the ether (2) occurred in 55% yield along with the formation of a small amount of the 1,3-dibenzyl derivative (*ca.* 7%). The site of monobenzylation (N-1 rather than at N-3) was proved from spectral data. It has been demonstrated [16,17] that in the u.v. spectra of uracils the peak shifts accompanying *N*-alkylation are characteristic of the site of the alkyl group. In our case, the differences of +23 and −2·5 nm in the $\lambda_{max.}$ values at pH 6·5 and 12·0 for compounds (2) and (3), respectively, were in excellent agreement with data predicted for (3) by the empirical rule of Nakanishi.[17] Structure (3) was further supported by n.m.r. data (see Experimental section). Mild acidic treatment of compound (3) gave the 1-benzyl-5-hydroxyuracil (4) in almost quantitative yield. The C(6)–NH_2 group was introduced by diazotisation, followed by reduction with dithionite. Conversion into



SCHEME

the monoacetate (7) was accomplished by the acetylation–deacetylation method described [6] for the preparation of the non-benzylated analogue (1c).

EXPERIMENTAL

N.m.r. spectra were recorded for solutions in [$^2H_6$]dimethyl sulphoxide unless otherwise stated.

5-Tetrahydropyran-2-yloxyuracil (2).—To a stirred mixture of ethyl formate (37 g), sodium hydride (13·5 g), and dry ether (*ca.* 500 ml), ethyl tetrahydropyran-2-yloxyacetate [15] (94 g) was added dropwise. After a short induction period (slight heating sometimes necessary at the beginning) an exothermic reaction started. Refluxing was controlled by cooling. After addition was complete stirring was continued for another 2 h, then urea (30 g) and absolute ethanol (450 ml) were added and the ether was distilled off. The remaining solution was heated under reflux for 15 h, then evaporated to dryness *in vacuo*. The residue was taken up in water; the solution was filtered, washed several times with ether, and made slightly acidic at 0° with glacial acetic acid. The precipitate was filtered off and washed with water to give the *tetrahydropyranyl ether* (28 g), m.p. >300°. Since heating during recrystallisation (from methanol or aqueous ethanol) caused loss of the tetrahydropyranyl group to some extent, the product was used in the next step without further purification.* Satisfactory spectral data were obtained after rapid crystallisation from hot methanol: δ 1·8 (6H, m, $[CH_2]_3$), 3·45—4·1 (2H, m, OCH_2), 5·3 (1H, m, O·CH·O), 7·35 (1H, s, 6-H), 10·6 [1H, N(1)H], and 11·3 [1H, N(3)H]; $\lambda_{max.}$ (pH 6·5) 272 (ε 5650), $\lambda_{max.}$ (pH 9·80) 275 (4130), $\lambda_{max.}$ (pH 12·0) 295 nm (3860); *m/e* 212 (M^+).

1-Benzyl-5-tetrahydropyran-2-yloxyuracil (3).—To a suspension of sodium hydride (2·4 g) in dry 1,2-dimethoxyethane (250 ml) a solution of the tetrahydropyranyl ether (2) (21·2 g) in the same solvent (200 ml) was added dropwise with stirring. Stirring was then continued for another 2 h, freshly distilled benzyl chloride (11·6 ml) was added, and the mixture was heated under reflux for 15 h. After cooling and removal of the solvent under reduced pressure, water was added; the mixture was acidified with glacial acetic acid and extracted rapidly with methylene chloride. From the aqueous layer unchanged starting material precipitated (8·4 g). The combined methylene chloride extracts were concentrated to a small volume, hexane was added until turbidity, and after cooling the *benzyl compound* was collected (9·5 g), m.p. 159—161° (from ethyl acetate–hexane); δ 1·45—1·95 (6H, m, $[CH_2]_3$), 3·7 (2H, m, OCH_2), 5·0 (2H, s, $PhCH_2$), 5·34 (1H, m, O·CH·O), 7·5 (5H, s, Ph), 7·8 (1H, s, 6-H), and 11·6br [1H, N(3)H]; $\lambda_{max.}$ (pH 6·5) 280 (ε 8460), $\lambda_{max.}$ (pH 12·0) 277 nm (6700) (Found: C, 63·5; H, 6·1; N, 9·6. $C_{16}H_{18}N_2O_4$ requires C, 63·6; H, 6·0; N, 9·3%).

From the mother liquor, after evaporation and chromatography of the residue on neutral alumina, as well as a second crop (0·5 g) of the monobenzyl derivative a small amount (1·6 g) of *1,3-dibenzyl-5-tetrahydropyran-2-yloxyuracil*, m.p. 90° (from methylene chloride), was isolated; δ 5·05 (2H, s, 1-$PhCH_2$), 5·15 (2H, s, 3-$PhCH_2$), 7·40 and 7·45 (10H, 2s, 2 × Ph), and 7·95 (1H, s, 6-H) (Found: C, 70·5; H, 6·0; N, 7·3%; M^+, 392. $C_{23}H_{24}N_2O_4$ requires C, 70·4; H, 6·2; N, 7·1%; *M*, 392·4).

1-Benzyl-5-hydroxyuracil (4).—To a hot methanolic solution of the ether (3) (10·0 g) a few crystals of naphthalene-β-sulphonic acid were added. Precipitation of the product soon began and the crystals were collected after prolonged cooling; yield 6·5 g, m.p. 239—241° (from methanol); δ 4·95 (2H, s, $PhCH_2$), 7·36 (1H, s, 6-H), 7·48 (5H, s, Ph), and 9·6—10·8br [2H, N(3)H and 5-OH] (Found: C, 60·8; H, 4·5; N, 12·95%; M^+, 218. $C_{11}H_{10}N_2O_3$ requires C, 60·5; H, 4·6; N, 12·8%; *M*, 218·2).

* In some of the batches, however, trituration with methanol was necessary to get rid of a by-product, presumably formed by self-condensation of ethyl tetrahydropyran-2-yloxyacetate, followed by reaction with urea.[15]

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1,3-*Dibenzyl-5-hydroxyuracil*.—This *material* was obtained from the dibenzyl tetrahydropyranyl ether as in the preparation of the monobenzyl compound (4); m.p. 120° (from methanol); δ 5·02 (2H, s, 1-PhCH_2), 5·16 (2H, s, 3-PhCH_2), 7·45 (11H, m, Ph and 6-H), and 8·9 (1H, s, 5-OH) (Found: C, 70·5; H, 5·25; N, 9·2%; M^+, 308. $C_{18}H_{16}N_2O_3$ requires C, 70·1; H, 5·2; N, 9·1%; *M*, 308·3).

1-*Benzyl-5-hydroxy-6-phenylazouracil* (5).—Aniline (4·48 g) in hydrochloric acid (15 ml) and water (30 ml) was diazotised at 5° with sodium nitrite (3·32 g) in water (*ca.* 20 ml). To the stirred, ice-cold diazo-solution, sodium acetate (11·85 g) was added, followed in portions by 1-benzyl-5-hydroxyuracil (4) (10·5 g) in 10% sodium hydroxide solution (40 ml). The mixture was then kept at low temperature for 15 h. The deep red *precipitate* was filtered off, washed with water, dried, and crystallised from nitrobenzene; m.p. 249—250° (yield almost quantitative) (Found: C, 62·8; H, 4·3; N, 17·55. $C_{17}H_{14}N_4O_3$ requires C, 63·35; H, 4·4; N, 17·4%).

6-*Amino*-1-*benzyl-5-hydroxyuracil* (6).—To a stirred hot aqueous suspension (75—85°) of the azo-compound (5) (1·0 g), small portions of sodium dithionite were added until the red colour had disappeared. The yellow suspension was cooled to 0° and the precipitate was collected, washed with water. and dried. The product (0·58 g) melted at 258° and was pure enough for further transformation. Recrystallisation from aqueous acetic acid and drying under high vacuum gave *material* of m.p. 269—270° (after sintering); δ 5·2 (2H, s, PhCH_2), 6·25 (2H, s, 6-NH_2), and 7·40 (5H, s, Ph) (Found: C, 56·7; H, 4·4; N, 17·4. $C_{11}H_{11}N_3O_3$ requires C, 56·65; H, 4·75; N, 18·0%).

6-*Acetamido*-1-*benzyl-5-hydroxyuracil* (7).—A mixture of compound (6) (0·5 g), acetic anhydride (6 ml), and anhydrous sodium acetate (0·27 g) was heated under reflux in nitrogen until a clear solution was obtained. The solution was cooled to room temp. and evaporated to dryness *in vacuo*. The residue was triturated with cold water and the solid was filtered off. Recrystallisation from methanol gave pure 6-*acetamido-5-acetoxy-3-acetyl*-1-*benzyluracil* (80—90%), m.p. 192—193°; δ 2·2 (6H, s, NH*Ac* and NAc), 2·3 (3H, s, OAc), 5·0 (2H, s, PhCH_2), 7·5 (5H, s, Ph), and 12·5br (1H, 6-NH) (Found: C, 56·7; H, 4·8; N, 11·7%; M^+, 359. $C_{17}H_{17}N_3O_6$ requires C, 56·8; H, 4·8; N, 11·7%; *M*, 359·3).

This triacetate (0·5 g) was dissolved with stirring in dilute sodium hydroxide solution and after cooling was acidified with cold dilute hydrochloric acid. The precipitate crystallised from aqueous methanol to give the *monoacetate* (7), m.p. 235°.

In an alternative procedure the triacetate was dissolved in ice-cold ethanol saturated with ammonia. The clear solution was set aside for 0·5 h and the precipitated monoacetate (7) was filtered off, washed with water, and dried under high vacuum. The yield was almost quantitative.

The compound is highly sensitive to air and rapidly develops a pink-red colour. For short periods it can be stored in high vacuum; δ 2·0 (3H, s, NAc), 5·0 (2H, s, PhCH_2), 7·4 (5H, s, Ph), and 8·8br, 9·8br, and 11·8br [3H, 5-OH, N(3)H, and 6-NH]; M^+, 275.

1-*Benzylconvicine Penta-acetate* (8).—6-Acetamido-1-benzyl-5-hydroxyuracil (7) (0·275 g) and a trace of α-naphtholphthalein were dissolved in an ice-cold 0·23N-sodium hydroxide (4·5 ml) in a nitrogen atmosphere. The blue solution was then added dropwise to tetra-*O*-acetyl-β-D-glucopyranosyl bromide (0·82 g) dissolved in dry acetone (9 ml) under nitrogen at 0° and with stirring. The pH of the resulting mixture soon dropped as indicated by the colour change to green. Dropwise addition of more 0·23N-sodium hydroxide (4·5 ml) kept the solution slightly alkaline. After addition was complete the mixture was set aside for 2 h and the cooled yellow solution was extracted with chloroform (3 × 5 ml). After the first extraction unchanged starting material (7) (0·14 g) was recovered from the water layer. The combined chloroform extracts were dried and evaporated under reduced pressure to give a mixture (0·6 g). Separation was achieved by careful chromatography on Florisil (60—100 mesh). Elution with benzene and with mixtures of benzene–chloroform of increasing polarity gave some unchanged tetra-acetyl-glucopyranosyl bromide and glucose tetra-acetate. *Compound* (8) (0·24 g) was eluted with pure chloroform followed by 3% methanol in chloroform; m.p. 120° [from methanol–water (1 : 9)]; δ ($CDCl_3$) 2·0—2·15 (15H, m, 5 × OAc), 3·5—5·5 (9H, m, glucose protons and PhCH_2), 7·3 (5H, s, Ph), 7·95br (1H, 6-NH), and 9·9br [1H, N(3)H] (Found: C, 53·05; H, 5·3; N, 6·9%; M^+, 605. $C_{27}H_{31}N_3O_{13}$ requires C, 53·5; H, 5·1; N, 6·9%; *M*, 605·5).

Convicine Penta-acetate (1d).—This compound was prepared by similar glycosidation of the monoacetate (1c) [6] (0·185 g), dissolved in ice-cold 0·23N-potassium hydroxide (5·3 ml) and added dropwise to a stirred solution of tetra-*O*-acetyl-β-D-glucopyranosyl bromide (0·822 g) in dry acetone (9 ml) in a nitrogen atmosphere. The solution was kept slightly alkaline (α-naphtholphthalein as indicator) by dropwise addition of more 0·23N-potassium hydroxide (4·5 ml). After 0·5 h the yellow solution was extracted with methylene chloride. From the dried organic layer a crude crystalline mixture was isolated which on recrystallisation from methanol–ether gave the *penta-acetate* (1d) (0·035 g), identical with the penta-acetate of natural convicine (m.p. and spectral data).

Convicine (6-*Amino*-5-β-D-*glucopyranosyloxyuracil*) (1a).—The penta-acetate (1d) (0·10 g) in dry methanol (5 ml) was treated with methanolic sodium methoxide solution (0·2N; 1 ml). The acetate slowly dissolved and the yellow solution was set aside overnight. The solvent was removed *in vacuo* and the residue crystallised from water. The crystals were identified as convicine by m.p., mixed m.p., and spectral data.

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