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UNCONVENTIONAL NUCLEOTIDE ANALOGUES—II
SYNTHESIS OF THE ADENYL ANALOGUE OF WILLARDIINE

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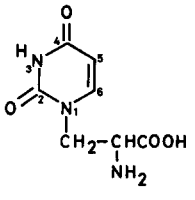
Abstract—The Michael addition of adenine (**6a**), 6-chloropurine (**6b**) and 2,6-dichloropurine (**6c**) to methyl α-chloroacrylate gives high yields of the corresponding adducts (**7a–7c**). Hydrolysis and amination of **7a** gave the adenyl analogue of Willardiine (**2**)

THE chemical quest for potential antimetabolites has focussed considerable attention on amino acid and peptide derivatives based on purine and pyrimidine systems of natural occurrence.[3] The discover of β-(N_1-uracilyl)alanine (Willardiine, **1**)[4] in nature has evoked special interest in the synthesis of its analogues in which the uracil moiety is exchanged for other pyrimidine derivatives. A Willardiine-mustard[5] has been recently synthesized in the anticipation of uncovering a new class of antitumor compounds.

In connection with a programme on "unconventional nucleotide analogues", underway in this laboratory, we have been engaged in investigating the chemistry of purines and pyrimidines, with a view to developing new and useful synthetic methods for attaining desired substitutions in these heterocyclic systems. Recent communications describing the synthesis of the adenyl analogue of Willardiine (**2**),[6] prompt us to report our own, and considerably simpler, approach for the preparation of **2**.



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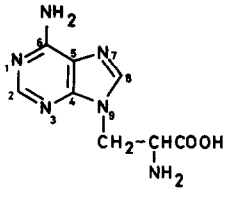
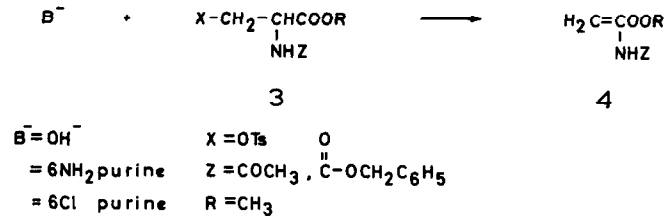


2

It has been pointed out by us earlier that simple nucleophilic substitution by purines of α-amino acids containing an activated carbon, does not – in the case of the corresponding alanine systems (e.g. **3**) – lead to significant amounts of the desired substituted products. The major course of the reaction is one of elimination, resulting in the formation of the α-aminoacrylic acid derivative **4** and its polymerization products. These observations are consistent with the reported behaviour of methyl N-carbobenzoxy-O-tosyl-serine, under influence of basic reagents.[8]

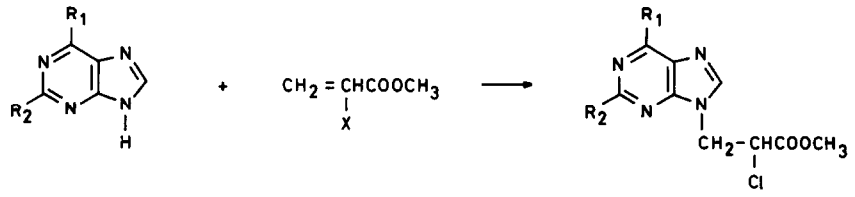


In considering other approaches for linking the N_9-position of a purine with a polyfunctional chain, the reported Michael additions of imidazole,[9] adenine[10] and 6-chloropurine[11] to electrophilic olefins (CH_2=CHCOOR, CH_2=CHCN) appeared to us to be of great potential interest for the synthesis of purinyl alanines. An added advantage of the latter approach was seen in the fact that both adenine and 6-chloropurine gave high yields of the desired N_9-*alkylated* product. Bearing in mind the nature of the transformations necessary to convert the alkyl residue to an α-amino acid, the α-halogenated acrylic acid derivatives were recognized as the suitable olefinic systems. The addition of phthalimide to methyl α-chloroacrylate has been described,[12] however, we are not aware of further attempts to synthesize substituted alanines via the aforementioned general sequence.

The attempted Michael addition of adenine to acrylic ester derivatives, under conditions described by Wieland *et al.*[9] for a similar reaction with imidazole, proved to be entirely unsuccessful in our hands. In orientation experiments designed to assess the relative suitability of the halogen in the acrylic ester (**5a** versus **5b**), it was observed that in case of **5b** the reaction proceeded extremely unsatisfactorily in the presence of catalytic amounts of added sodium methoxide—the normal reaction conditions for a Michael addition. This behaviour may be ascribed to the enhanced activity of bromine, in the Michael adduct, towards attack by methoxide ion – a reaction which consumes the base and thereby interrupts the cycle by which Michael addition proceeds.

The reaction of purine derivatives **6a**, **6b** and **6c** with methyl α-chloroacrylate (**5a**) gave excellent yields of the corresponding Michael addition products (**7a**–**7c**). In case of adenine (**6a**) sodium methoxide was used as the basic catalyst, while with the chloropurines (**6b** and **6c**) the best results were obtained when the reaction was carried out in dimethyl sulfoxide in presence of potassium carbonate. Conversion of **7a** to the Willardiine analogue was achieved in two steps involving acidic hydrolysis followed by amination of the halogenated acid **8a**.

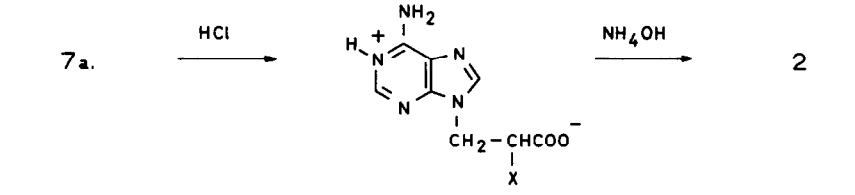
The isolation of pure **2** from the products of the amination reaction deserves some comment. As a rule, the amino acid is accompanied by small amounts of the starting chloro acid (**8a**) and the corresponding α-hydroxy acid (**8b**), from which it is difficult to separate. Use was made of the fact that at pH 8·5 both **8a** and **8b** were converted to their salts, while **2** was present entirely in the form of its zwitter ion. In practice, elution of the mixture from a silicagel column with an ethanol-NH_4OH mixture of pH 8·5 brought about a sharp separation of the amino acid (**2**) from its contaminants. The facile sequence of Michael addition of purines to α-chloro acrylic ester and conversion of the chloro compounds to α-amino acids provides a general approach to N_9-purinyl alanines. The conversion of **7b** and **7c** to hypoxanthinyl and guaninyl amino acids via conventional transformations is obvious, and is currently in progress.



6a: $R_1 = NH_2$ $R_2 = H$
b $R_1 = Cl$ $R_2 = H$
c $R_1 = Cl$ $R_2 = Cl$

5a: X = Cl
b X = Br

7a: $R_1 = NH_2$ $R_2 = H$
b. $R_1 = Cl$ $R_2 = H$
c· $R_1 = Cl$ $R_2 = Cl$



8a: X = Cl
b. X = OH

EXPERIMENTAL

All m.ps and b.ps are uncorrected. Analyses were carried out by Messrs. H. Pieters, W. J. Buis and W. van Duyl of the Microanalytical Department of this laboratory. IR, UV and mass spectra were recorded on Unicam SP 200, Cary-14 Recording and AE MS-9 Spectrometers respectively. NMR spectra (concentration 100 mg/0·5 ml, unless otherwise stated) were measured on a Varian Associates Model A-60 instrument.

Methyl α,β dichloropropionate[13]. Chlorine was passed into a mixture of 50 ml methyl acrylate and 2 ml DMF,[14] while the temp was maintained between 28–40°. In 2 hr the reaction was complete. The excess chlorine was pumped off under vacuum and the residual colourless liquid (100%) used directly in the following reaction; IR (neat) 1745 cm^{-1} (ester C=O); NMR ($CDCl_3$) δ 4·38–4·62, tr (1H, ClCHCOOMe), 3·6–4·2, m (2H, $ClCH_2$—) and 3·8 s (3H, OMe).

Methyl-α-chloroacrylate (**5a**)[15]. The ester was prepared following a modification of a procedure described by Marvel. A mixture of quinoline (24 g) α,β-dichloropropionate (16 g) and some hydroquinone was heated under a N_2 atmosphere during half an hour at 90°. The reaction mixture was distilled directly to give 8·6–11·1 g (70–90%) of **5a**, b.p. 55–66°/55 mm; IR (neat) 1715–1730 (ester C=O) and 1603–1625 cm^{-1} (C=C); NMR ($CDCl_3$) δ 6·02, d (1H, J = 2 c/s).

9-(β-*Carbomethoxy-β-chloroethyl*) *adenine* (**7a**). To a suspension of 8 g of adenine in 200 ml abs MeOH were added 200 mg of Na and the mixture was stirred under a N_2 atmosphere. After the disappearance of the metal 18 ml of methyl-α-chloroacrylate were added dropwise under continuous stirring. The mixture was refluxed for 5 hr after which only traces of adenine could be detected by means of TLC. [It is essential that a clear soln was obtained within an hour; if this was not the case some extra pieces of sodium were added]. The solvent was evaporated partially on the rotatory evaporator whereupon the adenine derivative **7a** crystallized out upon standing overnight at 0°. Recrystallization from MeOH provided the analytically pure sample, yield 9·1–13·6 g (60–90%); m.p. 168–169°; IR (KBr) 1730 (ester C=O), 1690, 1640 and 1600 (adenine nucleus); NMR (DMSO-d_6) δ 3·75, s (3H, $COOCH_3$), 4·6–5·4, m (3H, ABC system — CH_2—CH), 7·32, broad s (2H, NH_2) and 8·21, s (2H, adenine protons); Mol wt. (MS) 255·05229; Calc 255·05226;

UV (EtOH) 260 nm; (15,200). (Found: C, 41·3; H, 4·6; N, 25·8; Cl, 13·3; O, 14·8. $C_9H_{10}O_2N_5Cl.0{\cdot}5CH_3OH$ requires: C, 41·9; H, 4·45; N, 25·80; Cl, 13·05; O, 14·70%).

9-(β-*Carboxy*-β-*chloroethyl*)*adenine* (**8a**). 8·4 g of **7a** were dissolved in 250 ml 3N HCl and the soln refluxed for 3 hr. After cooling to 5° the mixture was brought to pH 8–9, with KOH pellets, (temp below 5°) which resulted in a clear soln. The pH was then rapidly adjusted to 3·5 with 1N HCl. Cooling to 0° and standing overnight gave white crystals of **8a** in 80–85% yield, m.p. 150° (dec); IR (KBr) 1690 cm^{-1} (COO^-); NMR (100 mg of acid and 100 mg of K_2CO_3 dissolved in 1·0 ml D_2O), δ 8·30 and 8·33, two s (C_3 and C_8 protons) and 4·8–5·1, m (3H, CH_2—CH); UV (pH 13) 260 nm (11,100). Mol. wt. (MS) 241.

9-dl(β-*Amino*-β-*carboxyethyl*) *adenine* (**2**). A soln of 1 g of **8a** in 38 ml of conc ammonia was heated at 105° for 70 hr in a Carius tube. After evaporation of the solvent the residual solid material was dissolved in a little ammonia and passed over a silicagel column (80 g) with a mixture of EtOH and conc ammonia as the eluent (conc ammonia was added to 96% EtOH until the pH was 8·5). The first fractions contained starting acid (**8a**). The adenine amino acid (**2**), contaminated with some NH_4Cl, remained after evaporation of the following fractions. Crystallization from water or eluting the amino acid from a cation exchanger wit ı 10% NH_4OH provided the pure amino acid. (Removal of Cl ion was achieved by washing the column beforehand with water until a negative $AgNO_3$ test was observed). Yield of the pure product m.p. 248–249 was 0·643 g (70%); IR (KBr) 1690 cm^{-1} (COO^-); UV 0·1N HCl 259 nm (13800), pH 7 261 nm (14200), 0·1N NaOH 261 nm (15100); NMR (100 mg of **2** + 100 mg of K_2CO_3 dissolved in 1 ml D_2O) δ 7·30 and 7·40, two s (2H, C_3 and C_8 protons) and 4·6–5, m (3H, CH_2—CH). (Found: C, 41·8; H, 4·8; N, 36·6. $C_8H_{10}N_6O_2.0{\cdot}5H_2O$ requires: C, 41·62; H, 4·79; N, 36·41%); Mol. wt. (MS) 222·08646; Calc. 222·08652. Significant peaks in the mass spectrum were observed at 177 (M − COOH) and 149 [M − CH(NH_2)COOH]; TLC (NH_4OH—EtOH, 1:30) R_f = for **8a** = 0·56.

2,6-*Dichloro*(β-*carbomethoxy*-β-*chlorethyl*) *purine* (**7c**). A mixture of 2·0 g **7c** (10·6 mmol), 1·6 ml methyl-α-chloroacrylate (about 14 mmol), 100 mg K_2CO_3 and 20 ml DMSO were stirred vigorously for 70 hr. After evaporation of the solvent (90°/1 mm rotatory evaporator) the residual oil was passed over a silicagel column with EtOAc as eluent. Crystallization from EtOAc provided light-yellow material, m.p. 110–115°, yield 1·64 g (50%); IR ($CHCl_3$) 1738 cm^{-1} (ester C=O); UV (EtOH) 274 nm and 250 nm (shoulder); NMR ($CDCl_3$) δ 3·86 s (3H, OMe), 4·7–5·5, m (3H, CH_2—CH) and 8·80, s (1H, C_8—H). Mol. wt. (MS) 307·96292: Calc. 307·96346.

6-*Chloro*-(β-*carbomethoxy*-β-*chloroethyl*) *purine* (**7b**). Treatment of **6b** with **5a**, according to the procedure described in the previous experiment, gave **7b** as an oil in 90% yield; IR ($CHCl_3$) 1738 cm^{-1} (ester C=O); UV (EtOH) 266 nm; NMR ($CDCl_3$) δ 8·28 and 8·75, two s (2H, C_3- and C_8- protons) and 4·7–5·1 m (3H, CH_2—CH). Mol.wt. (MS) 274·00242; Calc. 274·00195.

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