An unusual reduction path of chloropurine derivatives

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Summary. Reduction of 7- and 9-alkylchloropurines by sodium borohydride in methanol leads to 7,8-dihydropurines without affecting the chlorine atoms.

The classical route to 7-methylpurine involves reduction of 2,6-dichloro-7-methylpurine (**1**) by either Pd/C and H_2, or other reducing agents as HI and phosphorous derivatives[1]. However, yields are low, the working-up of the reaction mixture is tedious and the results are not reproducible. To circumvent these difficulties we sought for some better reducing agents. Usually, halopurines behave very similarly to alkyl halides by being susceptible towards nucleophilic displacements[2]. Since alkyl halides are efficiently reduced by sodium borohydride[3], it was anticipated that the latter reagent would reduce **1** similarly. Surprisingly, the reduction of **1** in boiling methanol took a completely different course to yield (40%) a new purine derivative to which the structure 2,6-dichloro-7-methyl-7,8-dihydropurine (**2**) is assigned on the following grounds:

a) The product (decomposing at about 187 °C after crystallization from benzene) gave a positive Beilstein test and analysed as $C_6H_6N_4Cl_2$[4]. Mass spectrum gave a molecular peak at m/e 205(M^+), i.e. addition of 2 mass units to **1**.

b) Product **2** ($pK_a \simeq 8$) had one ionisable proton and could be reprecipitated by acids from alkaline solutions.

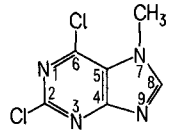
c) UV-spectrum of **2** (λ_{max} 317 nm, log ε 3.8 in MeOH) was similar to the open-chain analogue 4,5-diamino-2,6-dichloropyrimidine[5].

d) PMR in TFA (TMS as internal standard) showed 2 signals at δ 3.25 (s,3,CH_3) and δ 5.25 (s,2,CH_2). The starting purine **1** had (TFA) signals at δ 4.48 (s,3,CH_3) and δ 9.46 (s,1,CH). Thus, upon converting **1** to **2** the N-7 methyl group is shifted upfield indicating that the imidazole ring in **1** has lost its 'aromatic' character[6].

No reduced product was obtained from 2,6-dichloropurine and $NaBH_4$ in MeOH. In this case the purine sodium salt, which was rapidly formed, resisted any further nucleophilic attack of BH_4^-.

In order to explain the nucleophilic attack at C-8 in preference to any other position we have calculated the superdelocalisabilities for nucleophilic attack[7]. By using literature parameters[8] we find C-2 and C-6 to have an average value of 0.615 while C-8 showed the value of 0.716, i.e. more susceptible towards nucleophilic reagents.

Some other 7- and 9-alkyl-chloropurines behaved similarly to **1** upon reduction with BH_4^-. These reactions may open a new entry to the synthesis of compounds otherwise difficult to obtain.



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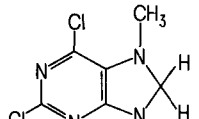

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4 All compounds gave satisfactory micro-analysis.
5 The dichloropyrimidine had λ_{max} 305 nm (MeOH). A similar difference in the UV-spectrum of a pyrimidine derivative and its 7,8-dihydropurine analogue has been reported previously and rationalized[6].
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Addition of sulphydryl groups to biliverdin

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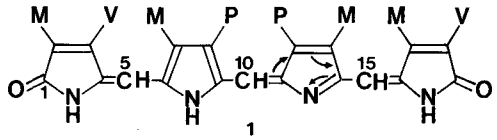
Summary. Sulphydryl groups add to the central methine bridge of biliverdin in organic solvents as well as in aqueous solution (pH 7.4). The addition reaction is favoured by albumin but reversed in acid media.

Biliverdin (**1**) has long been recognised as the intermediate in the physiological conversion of haem into bilirubin (**2**, X=H)[1-3]. Biliverdin does not occur in normal sera[4] but it can be detected in the serum of patients with biliary obstruction (re-oxidation of bilirubin)[5,6], hepatic disease[5-7] and malnutrition[8]. Reduction of biliverdin to bilirubin has been performed in vitro by a partially purified enzyme from guinea-pig liver[9-11], rat liver and kidney[12], and bovine spleen[13]. Nevertheless, considering the unusually pronounced electron acceptor properties of biliverdin[14,15], the possibility that part of it is reduced in vivo nonenzymatically cannot be excluded a priori. Particularly, one could presume that sulphydryl group-bearing compounds present in body tissues act directly as reductants of biliverdin[16], it being generally accepted (but not proven) that cysteine and glutathione rapidly reduce the green bile pigment[9]. We report here that sulphydryl groups do indeed interact with biliverdin in organic solvents as well as in aqueous solution: in each case examined, however, no bilirubin was formed but an adduct of the type **2** (X=SR).

When thioacetic acid dissolved in ethyl acetate (0.17 M, 1 ml) was added to a solution of biliverdin[17] in the same solvent (1.37×10^{-4} M, 250 ml), a rapid change of colour from blue to yellow was observed. After 15 min at room temperature the mixture was evaporated under reduced pressure and the residue dissolved in chloroform (5 ml) and added to hexane (10 ml). The resulting precipitate was shown to be pure compound **2** (X = $SCOCH_3$, 77% yield) on the basis of its elemental analysis and the following spectroscopic properties. The ^{1}H-NMR spectrum (0.05 M, $CDCl_3$, 100 MHz, TMS) exhibited all the signals of bilirubin, except for the singlet corresponding to the central methylene bridge[18], and 2 additional singlets at δ 2.38 (3H) and 6.72 (1H) due to the methyl of the thioacetyl group and to the proton at C-10 respectively. The base peak in MS appeared at m/e 286 in agreement with the typical cleavage of biladiene-a,c molecules at the central bridge giving rise to an oxodipyrromethene ion[19,20]; in addition, the peak at m/e 299, which is the most intense in bilirubin MS and corresponds to an oxodipyrromethene fragment bearing the C-10 methylene group[19], was absent. The visible spectrum in $CHCl_3$ displayed an absorption maximum at 446 nm, ε 48,200 (that of bilirubin being at 453–55 nm, ε 62,200)[21].



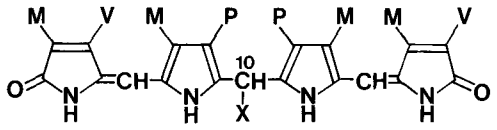


Fig. 1. Biliverdin (**1**), bilirubin (**2**, X = H) and biliverdin adducts (**2**, X = SR). M, methyl; V, vinyl; P, propionic chain.

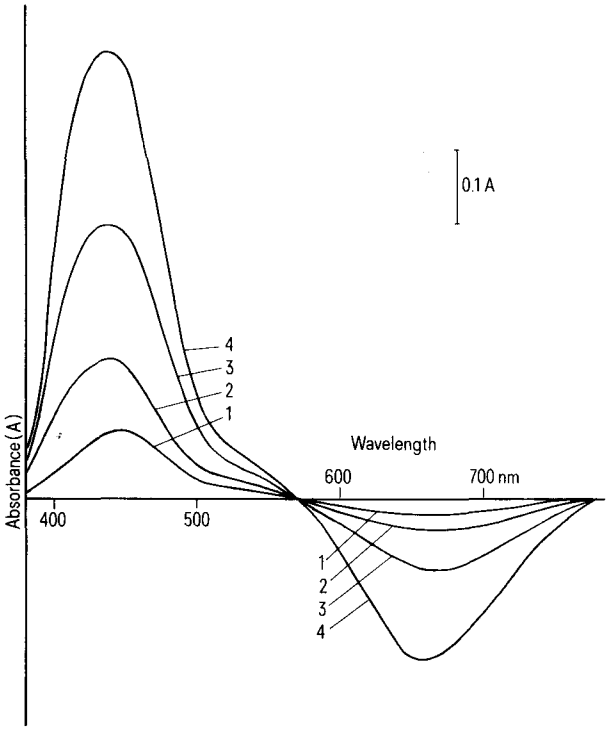


Fig. 2. Visible difference spectra of the reaction of biliverdin with 2-mercaptopropionylglycine (MPG) at 3 °C. Reference beam: biliverdin solution 7.7×10^{-5} M in phosphate buffer 0.05 M, pH 7.4 (cell path, 1 cm). Sample beams: reference solution containing MPG (1) 3.85×10^{-4} M; (2) 7.70×10^{-4} M; (3) 1.54×10^{-3} M; (4) 3.85×10^{-3} M.

In an analogous way, treatment of biliverdin with methyl mercaptan and with methyl thioglycolate afforded the expected adducts **2** [X = SCH_3: 74% yield, singlets at δ 2.16 (3H, methylthio group) and at δ 5.72 (1H, C-10 proton), γ_{max} 447 nm (ε 53,800), m/e 286 (100%); X = $SCH_2CO_2CH_3$: 54% yield, singlets at δ 3.21 and 3.73 (2H and 3H respectively, $SCH_2CO_2CH_3$) and at δ 6.18 (1H, C-10 proton), λ_{max} 445 nm (ε 52,400), m/e 286 (100%)], thus indicating that the addition of SH-groups to the central methine bridge of biliverdin is quite a general reaction. This fact can be rationalized in terms of π-electron density[14,15,22] and HSAB principle[23], i.e., a soft base such as the sulphydryl group binds easily to a soft electrophilic site[24] (see arrows in figure 1). The addition reaction is rapidly reversed in acid media, as proved by the almost quantitative recovery of biliverdin from solutions of the above adducts **2** in chloroform containing 0.1% trifluoroacetic acid.

It was found that sulphydryl compounds, such as 2-mercaptopropionylglycine (Thiola®), cysteine, glutathione and reduced lipoic acid, do not reduce biliverdin in aqueous solution (phosphate buffer 0.05 M pH 7.4, 7.7×10^{-5} M with respect to the pigment, in an argon atmosphere at 0 to 25 °C) but quickly add to it as in organic solvents. In fact, the starting biliverdin was recovered just after acidification of its solution made yellow by addition of a single SH-reagents, while bilirubin gave no verdinoid product under the same conditions and in the presence of the corresponding disulphide. Further evidence from spectrophotometric measurements (figure 2) was that the nucleophilic addition is an equilibrium reaction, the equilibrium being displaced to the rubinoid product as temperature is lowered.

It is noteworthy that the addition reaction appears to be strongly favoured in human serum albumin solution (HSA) as well as in normal sera: e.g., a molar ratio of 2-mercaptopropionylglycine to biliverdin 5:1 in HSA (AB Kabi, Stockholm, 5%, pH 7.4 at 10 °C) is enough to produce yellow colour with complete disappearance of biliverdin.

The bilirubin derivatives reported here were found to react readily with 2-ethoxycarbonylbenzenediazonium fluoborate in chloroform-methanol, with the exception of the thioacetyl derivative which turns back immediately to biliverdin in the presence of methanol; they gave rise to the same mixture of dipyrrolic azopigments as bilirubin[25], i.e., a vinyl/isovinyl isomer ratio 1:1 in TLC[26]. They were also shown to be diazo-positive when dissolved or prepared in albumin solution. This behaviour of biladienes **2** (X = SR), together with their electronic absorption features and their rapid reversion to biliverdin in acid media (particularly on silicagel plates), makes them practically indistinguishable from natural bilirubin or biliverdin by means of the common methods used for bile pigment analysis[27–30]. We therefore conclude that the addition of SH-groups to biliverdin could occur in vivo, but it might have passed unnoticed for the above-mentioned reasons.

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New norsesquiterpene aldehyde and sesquiterpene hemiacetal from the seed of *Polygonum hydropiper*

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Summary. A new norsesquiterpene aldehyde, polygonal and a new sesquiterpene hemiacetal, isodrimeninol having drimane skeleton have been isolated from the seed of *Polygonum hydropiper,* and their structures have been established to be **1** and **5.** Polygonal showed pungency and plant growth inhibitory activity.

The leaf of the medicinal plant, *Polygonum hydropiper* (Polygonaceae), contains the intense pungent sesquiterpene, polygodial (**10**)[1,2]. The seed of this plant also shows the slightly different pungency from that of the leaf. We have now investigated the chemical constituents of the seed and a new norsesquiterpene aldehyde, polygonal (**1**) and a new sesquiterpene hemiacetal, isodrimeninol (**5**) have been isolated together with the previously known polygodial (**10**), isopolygodial (**11**) and confertifolin (**12**). Column chromatography and preparative TLC on silica gel of the crude extract of the ground seed of *P. hydropiper* resulted in the isolation of **1** (1.6%, total wight of the extract), **5** (2%), **10** (18%), **11** (5.5%) and **12** (2.2%).

Polygonal (**1**), $C_{14}H_{22}O_2$ (M^+ 222), m.p. 116–117 °C, $[\alpha]_D$ −7.3° (c, 7.4 in $CHCl_3$), showed the presence of an α,β-unsaturated aldehyde group [2740, 1675 cm^{-1}; 223 nm (log.ε, 4.14), 2,4-DNP, m.p. 139–140 °C; 370 nm (log.ε, 4.27)], a hydroxyl group (3400 cm^{-1}) and a double bond (1630 cm^{-1}). The NMR-spectrum contained the signals of three tertiary methyl groups (0.93, 1.00, 1.06 ppm, s, 9H), two of which might be gemdimethyl group (1385 and 1375 cm^{-1}), a vinylic proton (6.53 ppm, s) assignable to $-CH-C{=}CHO$ and a carbinyl proton (4.66 ppm, bs, $W_{1/2}$ = 7 Hz) and an aldehyde proton (9.50 ppm, s). The above spectral data, coupled with the molecular formula, showed that **1** was a bicyclic norsesquiterpene aldehyde with a hydroxyl group. The arrangement of B-ring of **1** is apparent from the following arguments. Acetylation of **1** with acetic anhydride-pyridine gave a monoacetate (**2**) [$C_{16}H_{24}O_3$ (M^+ − 60, 204); 1730, 1245 cm^{-1}; 2.00 ppm (s, 3H), 5.57 ppm (bs, $-CHOAc$)] and a small amount of dehydrated product (**4**) [$C_{14}H_{20}O$ (M^+ 204); 1683 cm^{-1}], indicating that **1** possessed a secondary alcohol. Oxidation of **1** with Collin's reagent afforded **3** [$C_{14}H_{20}O_2$ (M^+ 220); 10.03 ppm (s, CHO)]. The stereochemistry of the secondary hydroxyl group was confirmed to be α on the basis of the broad singlet ($W_{1/2}$ = 7 Hz) of H-7[3] and the facile formation of **4** from **1**. These results and the negative Cotton effect (235 nm, $\Delta\varepsilon$, −9.96; 330 nm, $\Delta\varepsilon$, −2.52), along with the co-occurrence of the other drimane type sesquiterpenes, **10,**