X-Ray Molecular Structure of Caissarone, a Novel Purine Derivative from the Sea Anemone *Bunodosoma caissarum* Correa 1964†

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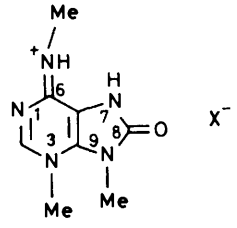
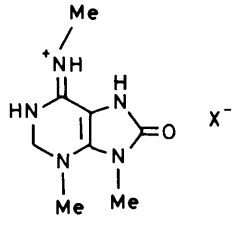
A new quaternary purine derivative isolated from the sea anemone *Bunodosoma caissarum* has been identified, by spectroscopic measurements and *X*-ray diffraction analysis, to be 3,9-dimethyl-6-methylimino-8-oxo-3,6,8,9-tetrahydropurine (caissarone) and isolated as the hydrochloride. The *X*-ray structure indicates that the exocyclic nitrogen is apparently the most electron-rich centre and that the molecule is planar.

Biologically active factors such as neuro- and cardio-toxic principles, haemolytic toxins, and proteinase inhibitors have been found in sea anemones, both in nematocyst tentacles and in whole animals.[1] *Bunodosoma caissarum* Correa 1964 (Anthozoa, Actiniaria) is a sea anemone abundant on the reefs off the Brazilian coast.[2] Animal homogenates and materials released from nematocyst discharges were shown to be active on cholinoceptive preparations.[3] A cardiotoxic and CNS-acting polypeptide, bunodosine, has been found in a methanolic extract of this organism,[4] from which we have now isolated a novel purine derivative which we have named caissarone, isolated as its hydrochloride (**1**).



(1) X = Cl
(2) X = Picrate
(3) X = OH



(4) X = Picrate

The material from an acetone extract of whole animals was triturated with methanol, to yield a white precipitate, which was purified by repeated crystallizations in water–methanol to give compound (**1**) as needles, m.p. 285—290 °C. The product gave a positive Dragendorff test,[5] and was characterized as a quaternary ammonium salt. Its structure was established by detailed n.m.r. spectroscopy and *X*-ray diffraction analysis.

Caissarone hydrochloride (**1**) has i.r. absorption bands at 3 450, 3 200, 1 680, and 1 610 cm^{-1}, due to OH or NH, $CONH_2$, C=N, and C=C groups respectively. The mass spectrum shows a molecular ion at *m/z* 193 for $C_8H_{11}N_5O$. In the ^1H n.m.r. spectrum, measured in D_2O, one methine singlet is observed at δ_H 8.20, characteristic for a proton at C-2 or C-6 of a pyrimidine ring, or at C-8 of the imidazole ring in purines. The signals at higher field, δ_H 4.20, 3.74, and 3.17, which are singlets for 3 H each, disclosed the presence of three *N*-methyl groups. The ^{13}C n.m.r. spectrum revealed that caissarone hydrochloride (**1**) contains 8 carbon atoms, as shown by the presence of four singlets, one doublet, and three quartets, the latter signals being assigned again to the *N*-methyl groups (Table 1). Comparison of these data with those reported for various purines,[6–9] as well as with the ^1H n.m.r. spectrum, strongly suggests that caissarone hydrochloride (**1**) contains a purine skeleton, corresponding to the formula $C_5H_3N_4$, with additional nitrogen, oxygen, and three methyl groups. Furthermore, the presence of an amide group, inferred from the i.r. band at 1 680 cm^{-1}, was confirmed by a ^{13}C n.m.r. signal at δ_C 153.8. From the spectral data several possible isomers could be suggested. Since the product was found to be stable and produced good single crystals, an unequivocal structural determination was carried out using *X*-ray diffraction analysis as shown in the Figure.

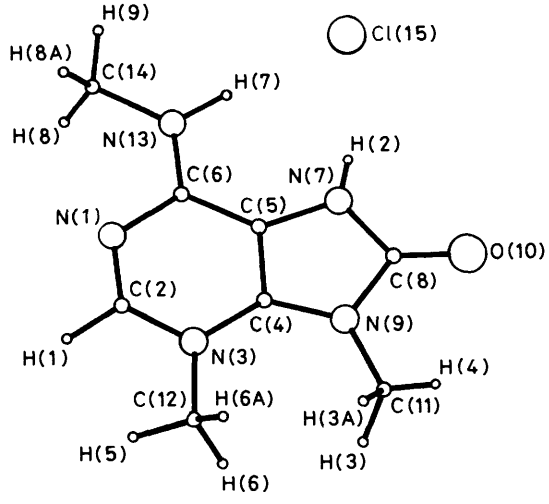


Figure. Molecular structure of caissarone hydrochloride (**1**)

Caissarone hydrochloride (**1**) had an unexpected 3,9-dimethyl-6-methylimino-8-oxo-3,6,8,9-tetrahydropurine hydrochloride formula. The molecule displays a crystallographic mirror plane of symmetry and therefore is completely planar. Moreover, the *X*-ray structure clearly shows that the extra hydrogen (from the hydrogen chloride) is attached to the exocyclic N (N-13), which probably means that this is the most electron-rich centre in the conjugated fragment N–C=N–C=NH–CH_3 of caissarone. This situation indicates that a distribution of double bonds as shown in structure (**1**) makes an important contribution to the resonance structure, and

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Table 1. ^{13}C N.m.r. data of compounds (**1**), (**3**), and (**4**)

Carbon	(1)[a]	(3)[a]	(4)[b]	6-Methyl-aminopurine[8]
2	148.2 (d)	150.0 (d)	61.0 (t)	152.4
4	138.5 (s)	137.9 (s)	147.3 (s)	150.0
5	105.6 (s)	117.0 (s)	93.2 (s)	118.2
6	149.1 (s)	143.7 (s)	148.8 (s)	154.7
8	153.8 (s)	161.6 (s)	161.0 (s)	138.7
NCH_3	28.8 (q) 30.6 (q) 39.8 (q)	28.4 (q) 30.2 (q) 39.3 (q)	28.0 (q) 28.7 (q) 36.4 (q)	27.2

Spectra taken in: [a] D_2O. [b] $(CD_3)_2SO$.

probably causes the planarity of the molecule. One can also see in the Figure that the counterion Cl^- is located in the neighbourhood of the charged methyl iminium moiety, supporting the fact that the proton is attached to the exocyclic nitrogen. This formula easily accounts for the spectral data, particularly the unusual low-field signal of the methyl linked to the quaternary iminium nitrogen.[10] Furthermore, it is interesting to note that no hydrogen bonding was detected in the crystal structure of caissarone hydrochloride.

Caissarone gave a picrate (**2**), m.p. 245—250 °C, and the hydrochloride was converted by resin exchange into the hydrate (**3**), m.p. 280—285 °C. Treatment of compound (**1**) with $NaBH_4$ afforded the 1,2-dihydro derivative, obtained as the picrate (**4**), m.p. 214—217 °C, which displayed a ^{13}C n.m.r. spectrum with four singlets, one triplet, and three quartets (Table 1), beside resonances for the picric anion.

Purine bases are widely distributed in marine organisms and a few non-classical derivatives have been isolated, among them the potent neurotoxin saxitoxin.[11] Recently two novel quaternary 9-methyladenine salts of diterpenes with antimicrobial activity, ageline A and B, have been described.[12] Caissarone represents a further example of a purine with an uncommon structure characterized by a methylimino group at position 6 and a carbonyl at C-8. This unusual structure is, in effect, a dihydropyrimidine ring fused to a cyclic urea.

The caissarone salt (**1**) has been found to be inactive in the Leukaemia screen (3PS31) tests at dose levels up to 200 mg kg^{-1}. These data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland.

Experimental

M.p.s were taken on a Kofler–Reichert hot-stage microscope and are uncorrected. U.v. spectra were recorded on a Zeiss-DMR 21 spectrophotometer for solutions in methanol, and i.r. spectra on a Perkin Elmer 727-B instrument for KBr pellets. ^1H and ^{13}C n.m.r. spectra were taken on a Varian EM-360 (at 60 MHz) and FT-90A (at 20 MHz) instrument respectively with tetramethylsilane as internal standard. Coupling constants are in Hz. Microanalyses were performed in the microanalytical laboratories of the Institute of Chemistry, University of São Paulo, and of the Weizmann Institute. The mass spectra are from the University of Ottawa, and from the Department of Chemistry, the University of Rio de Janeiro (NPPN), and the high-resolution spectra are from the Weizmann Institute Chemical Services.

Collection, Extraction, and Isolation.—*Bunodosoma caissarum*, a red-violet anemone, was collected on the reefs (3 m depth) off the São Sebastião coast, State of São Paulo, and frozen for transport. The minced animals (1 300 g) were extracted with three portions (3 l each) of acetone, the combined extracts (acetone–water) were filtered, and the organic phase was evaporated off. The aqueous concentrate was first treated with diethyl ether to remove pigments, lipids, and sterols, and the aqueous phase was then lyophilized to a viscous paste, which was triturated in methanol to obtain a white precipitate. The material (3.2 g, 0.24%) recrystallized from aqueous methanol to give caissarone hydrochloride (**1**).

Table 2. Atom co-ordinates ($\times 10^4$) with e.s.d.s in parentheses

	x	*y*	*z*
N(1)	917(2)	2 356(2)	0
C(2)	736(2)	3 060(2)	0
N(3)	1 213(2)	3 640(2)	0
C(4)	1 950(2)	3 464(2)	0
C(5)	2 175(2)	2 737(2)	0
C(6)	1 645(2)	2 164(2)	0
N(7)	2 936(2)	2 736(2)	0
C(8)	3 195(2)	3 444(2)	0
N(9)	2 563(2)	3 907(2)	0
O(10)	3 839(2)	3 663(2)	0
C(11)	2 641(4)	4 715(2)	0
C(12)	920(3)	4 406(2)	0
N(13)	1 830(2)	1 449(2)	0
C(14)	1 285(3)	845(2)	0
Cl(15)	3 620(1)	1 137(1)	0

Caissarone Hydrochloride, 3,9-*Dimethyl*-6-*methylimino*-8-*oxo*-3,6,8,9-*tetrahydropurine Hydrochloride* (**1**).—White *needles*, m.p. 285—290 °C (from aqueous MeOH); *m/z* 193 (M^+, 22%); $\lambda_{max.}$ 228 (ε 21 600) and 302 nm (17 710) (Found: Cl, 15.3%, $M^+ - HCl$, 193. $C_8H_{12}ClN_5O$ requires Cl, 15.43%; *M*, 229.5).

Caissarone Picrate (**2**).—A saturated alcoholic solution of picric acid was added to an aqueous solution of caissarone·HCl (**1**) until complete precipitation of the *picrate*, m.p. 245—250 °C (from EtOH); δ_H [$(CD_3)_2SO$] 8.5 (s, 3 H) 8.2—7.8 (br, $w_{\frac{1}{2}}$ 9, 1 H), 4.1 (s, 3 H), 3.60 (s, 3 H), and 3.05 (d, *J* 2.5, 3 H) (Found: C, 39.8; H, 3.4; N, 26.6. $C_{14}H_{14}N_8O_8$ requires C, 39.81; H, 3.34; N, 26.54%).

Caissarone Hydrate (**3**).—An aqueous solution of the hydrochloride (**1**) was eluted through an ion-exchange column of Amberlite CG-400 and concentrated under reduced pressure to give the *hydrate* (**3**), m.p. 285—290 °C (from MeOH); $\nu_{max.}$ 3 250, 1 660, 1 620, 1 480, 1 430, 1 325, 1 280, 1 200, 1 060, and 920 cm^{-1}; δ_H (D_2O) 8.18 (s, 1 H), 4.20, (s, 3 H), 3.64 (s, 3 H), and 3.10 (s, 3 H) (Found: C, 38.6; H, 6.1. $C_8H_{13}N_5O_2{\cdot}2H_2O$ requires C, 38.83; H, 6.92%); h.r.m.s. (Found: M^+, 193.0964. $C_8H_{11}N_5O{\cdot}H_2O$ requires $M - H_2O$, 193.0963).

1,2-*Dihydrocaissarone Picrate* (**4**).—A solution of compound (**1**) (100 mg) and $NaBH_4$ (100 mg) in water was stirred for 3 h. After neutralization with dil. HCl, the solution was treated with saturated alcoholic picric acid to give the *picrate* (**4**), m.p. 214—217 °C (from EtOH); $\nu_{max.}$ 3 250, 1 710, 1 640, 1 610, 1 540, 1 480, 1 340, 1 310, 1 260, 1 145, 1 060, and 890 cm^{-1}; δ_H [$(CD_3)_2SO$] 9.7 (br, 1 H, NH), 8.50 (s, 2 H), 8.1 (br, 1 H, NH), 4.46 (s, 2 H), 3.10 (s, 3 H), 2.90 (s, 3 H), and 2.86 (d, *J* 2, 3 H) (Found: C, 40.3; H, 3.7; N, 26.15. $C_{14}H_{16}N_8O_8$ requires C, 39.62; H, 3.80; N, 26.41%). Picric anion: δ_c [$(CD_3)_2SO$] 152.2 (C-1), 141.9 (C-4), 125.4 (C-3 and -5), and 124.6 (C-2 and -6).

Crystal Data for Caissarone Hydrochloride (**1**).—$C_8H_{12}ClN_5O$, *M* = 229.5. Tetragonal, *a* = 17.972(1), *b* = 17.972(1), *c* = 6.688(1) Å, *V* = 2 160 Å^3 (by least-squares refinement on

diffractometer angles for 25 automatically centred reflections, $\lambda = 1.5418$ Å), space group $I4/m$ (No. 87), $Z = 8$, $D_x = 1.41$ g cm^{-3}. Transparent six-sided prisms.

Data Collection and Processing.—CAD4 diffractometer, $\omega/2\theta$ mode with ω scan width $= 0.80 + 0.14 \tan\theta$, constant ω scan speed 3.3 deg min^{-1}, Ni-filtered Cu-K_α radiation; 833 unique reflections measured ($2° < \theta < 70°$, h,k,l), giving 829 reflections with $F_o > 3\sigma(F_o)$.

Structure Analysis and Refinement.—Direct methods followed by full-matrix least-squares refinement with all non-hydrogen atoms anisotropic and hydrogens (found from a difference Fourier map) isotropic. The weighting scheme, $w = 0.988/[\sigma^2(F_o) + 0.000\,30F_o^2]$, with $\sigma(F_o)$ from counting statistics, gave satisfactory agreement analyses. Final R and R_w values are 0.040 and 0.047. All calculations were performed with the SHELX-76 package of crystallographic programs.[13] Fractional atomic co-ordinates for non-hydrogen atoms are in Table 2. Non-hydrogen atom anisotropic temperature factors, isotropic temperature factors for H atoms, and bond lengths and angles are deposited as Supplementary Publication No. SUP 56641 (3 pp.).*

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