reaction and therefore is useful to determine the most feasible sites of protonation.[29-31]

Molecular electrostatic potential minimum energies on N1 and N3 atoms are displayed in Table III. Of particular note is the poor ability of both STO-3G and 3-21G basis sets to provide quantitatively correct values of the MEP minimum energy, confirming our previous suggestions that the inclusion of polarization functions is necessary to obtain reliable MEP energy values.[24,32] Nevertheless, for this molecule, from a qualitative point of view all the methods provide similar results. Thus irrespective of the wave function employed, the N3 MEP minimum is deeper than the N1 one, the difference varying from 6.4 (STO-3G) to 9.7 kcal/mol (3-21G) for the N7–H tautomer and between 2 (STO–3G) and 6.9 kcal/mol (3-21G) for the N8–H tautomer. MEP minima on N1 and N3 for the N8–H tautomer are deeper than those of the N7 tautomer. These results agree with the protonation energy values shown in Table II and clearly demonstrate that the N3 atom is the most basic point in either N7–H or N8–H tautomeric forms of the aminopyrazolo molecule and that the less stable N8–H tautomer is more basic than the N7–H tautomer, in agreement with the suggestions of Dodin et al.[15]

Discussion

The main objective of this work has been the study of the intrinsic tautomeric and acid/base characteristics of the 7-aminopyrazolopyrimidine molecule. However, the interest of extrapolating the conclusions obtained here to the formycin molecule and other derivatives is obvious. NMR results reported by Chenon et al.[14] clearly demonstrated that the tautomerism of the 7-aminopyrazolopyrimidine derivatives is not related to the nature of the 9-substituent group (3-substituent group according to IUPAC nomenclature). This leads us to the suggestion that the conclusions obtained here about the tautomerism of the 7-aminopyrazolopyrimidine are also valid for the formycin molecule. No experimental studies on the role of the ribose moiety in the acid/base characteristics of the 7-aminopyrazolopyrimidine exist to our knowledge. Available data on the acid/base properties of adenosine and adenine[33] show that the presence of the ribose causes a small effect on the pK_a value of adenine, both adenine and adenosine being protonated at the N1 atom. Accordingly, for the formycin molecule, it has been suggested that the ribose moiety causes a slight decrease in the basicity of both N1 and N3 and that this decrease is greater for the N3 atom, especially if the ribose moiety is in the syn conformation around the glycosidic bond.[9]

Our ab initio quantum chemical calculations point out that the N7–H is the most feasible tautomer of the neutral aminopyrazalopyrimidine molecule, in excellent agreement with experimental data of formycin.[8,13-16] The calculated energy difference between the N7–H and N8–H tautomers is about 2 kcal/mol (at the MIXED 1 level), in good agreement with the value of 1 kcal/mol reported by Dodin[15] for the formycin molecule. The protonation of the molecule at either N1 or N3 atoms produces a drastic change in the tautomeric perference from the N7–H to the N8–H tautomer. It should be noted that the N8–H—N7–H energy difference is notable for the N1-protonated form but smaller for the N3-protonated form. These facts explain why Koyama et al.[11] found for the N1-protonated formycin that the N8–H tautomer was present, while McKenna et al. for the N3-protonated 3′-deoxyformycin found that the change from the N7–H to the N8–H did not occur.[7]

Experimental results[8] point out the existence of both N1 and N3 protonated forms of formycin in solution. Our theoretical calculations suggest that the N3 atom is more basic than the N1 one (in agreement with the results reported by McKenna et al.[7]) and that the difference in basicity is more notable for the N7H tautomer than for the N8H tautomer. The presence of N1-protonated form in the crystal structure reported by Koyama et al.[11] can be due to the low energy difference in the basicity of N1 and N3 for the N8–H tautomer and to the effect of the ribose moiety as discussed above.

Both N7–H and N8–H tautomers of 7-aminopyrazolopyrimidine derivatives can exist in either neutral, N1-protonated, or N3-protonated forms. Theoretical results suggest that in the *gas phase* N3 is the most basic atom of the molecule. N7–H is the most stable tautomeric form for the neutral molecule, while N8–H is the preferred tautomer for protonated molecule. It is also interesting to note that the neutral N8–H tautomer is a stronger base than the neutral N7–H tautomer. Results presented in this paper are consistent with the data existing for neutral formycin (see ref 8, 10, and 12–16). Also, references about the increase in basicity when formycin changes from N7–H to N8–H tautomeric form have been reported.[15] Finally, it should be stressed that the most stable structure found by quantum chemical calculations for the protonated 7-aminopyrazolopyrimidine molecule is different from either Koyama's[11] nor McKenna's[7] crystal structures.

Our quantum mechanical calculations have been performed within the Hartree–Fock framework, and no large extended basis set has been used due to the computational limitations derived from the size of the molecules studied. Nevertheless, since qualitatively similar results are obtained from the different basis sets, it can be suggested that the extension of the basis set and the inclusion of correlation effects would not introduce significant variations in the conclusions obtained.

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A Very Convenient Synthesis of Cyclopenta[*cd*]pyrene

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Cyclopenta[*cd*]pyrene (CPP, **5**), a constituent of the class of the polycyclic aromatic hydrocarbons, occurs in a wide variety of carbon black soots,[1-9] in cigarette smoke,[10]

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and in car engine exhaust gases.[2,3,11] CPP has been shown to be a potent bacterial mutagen,[12] and it is carcinogenic on the skin and in the lungs of mice.[13-15]

Studies on the biological activity and metabolism of CPP and its derivatives require relatively large amounts of CPP. Since isolation of CPP from soot is possible only in small quantities, several syntheses have been developed.[16-21] In all these methods pyren-4-ylacetic acid (or its 4,5-dihydro derivative[21]) is synthesized and then converted into the ketone cyclopenta[*cd*]pyren-3(4*H*)-one (CPP-3(4*H*)-one, **3**).

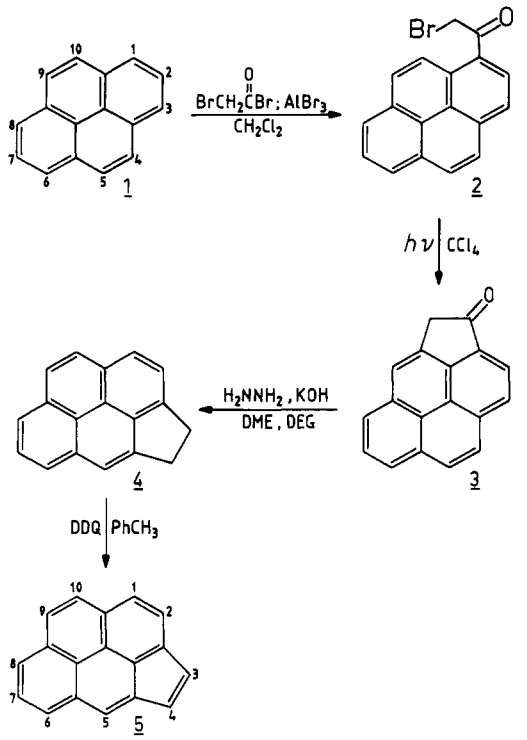
It has been reported that it is essential to start with the 4-substituted pyrene derivative since the more readily accessible pyren-1-ylacetic acid cannot be cyclized to the ketone CPP-(3*H*)4-one.[16,18,21] Pyren-4-ylacetic acid cannot easily be synthesized directly from pyrene, and the majority of the methods therefore start with the more expensive 1,2,3,6,7,8-hexahydropyrene.[16-20] The cyclization of the pyren-4-ylacetic acid is, however, difficult in spite of the high reactivity of position 1. Vigorous conditions such as treatment with the hazardous liquid HF are required.

These problems can be avoided by using a photochemical ring closure of an α-halo ketone as described by Matsumoto et al.[22] In contrast to the situation in the ground state, position 4 of pyrene appears to be reactive in the excited state. This permits the use of a 1-substituted derivative of pyrene, which can then easily be cyclized. A simple Friedel–Crafts acylation affords 1-(bromoacetyl)-pyrene (**2**) which can be converted to CPP-3(4*H*)-one by irradiation.

The Friedel–Crafts acylation of pyrene with bromoacetyl bromide in dichloromethane in the presence of $AlBr_3$ affords 1-(bromoacetyl)pyrene in 72% yield. Upon irradiation of a solution of 1-(bromoacetyl)pyrene in carbon tetrachloride, the pure CPP-3(4*H*)-one is isolated after chromatography in 39% yield (45% conversion).

To synthesize CPP, CPP-3(4*H*)-one was first reduced by the Huang-Minlon modification of the Wolff–Kishner reaction, and the product was then oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), as described by Tintel et al.[21] The last two steps both yield 95%.

Scheme I. Synthesis of Cyclopenta[*cd*]pyrene



The photochemical route (Scheme I) is very suitable for small scale production of CPP and can also be very useful in the synthesis of substituted CPPs because it uses standard laboratory procedures and is cheap, efficient, and safe.

Experimental Section

General Procedures. Pyrene was purchased from Janssen Chimica (99+%) and used without further purification. Bromoacetyl bromide, aluminum bromide, and lithium carbonate were commercial products and were used without further purification. All solvents were distilled before use. Silica (230–400 mesh) ASTM was supplied by Merck. Irradiation was carried out in a well-stirred solution in a vessel fitted with a Pyrex inner tube in which the light source, a Hanau TQ 150 medium-pressure mercury arc, was mounted. The inner tube and the outside of the vessel were cooled with water.

1-(Bromoacetyl)pyrene (2). $AlBr_3$ (5.34 g, 20.0 mmol) was added over 45 min in small portions to a solution of pyrene (2.00 g, 10.0 mmol) and bromoacetyl bromide (0.87 mL, 10 mmol) in 100 mL of dichloromethane. The temperature of the mixture was kept between −5 and 0 °C. After stirring at room temperature for 22 h, the mixture was poured onto ice. The dichloromethane layer was subsequently washed with a saturated aqueous sodium bicarbonate solution and with water and finally dried over magnesium sulfate. After the solution was treated with activated coal, during which the initially brown color changed to yellow, the solvent was evaporated. The crude product was adsorbed on a small amount of silica, and this material was chromatographed with hexane/dichloromethane (1:3), yielding 2.33 g (7.2 mmol, 72%) of 1-(bromoacetyl)pyrene as a yellow solid. The compound was recrystallized from dichloromethane/cyclohexane, yielding 1.97 g of yellow needles (6.1 mmol, 61%), melting point 128–129 °C: ^{1}H NMR (100 MHz, $CDCl_3$, TMS) δ 4.63 (s, 2 H, α-CH_2), 7.80–8.36 (m, 8 H, H(2-9)), 8.84 (d, 1 H, H(10), J = 9.3 Hz); MS (50 °C), m/z (rel intensity, fragment) 324/322 (20, M^+), 229 (100, $M^+ - CH_2Br$), 201 (57, $M^+ - COCH_2Br$); UV (CCl_4) λ_{max} (rel ϵ) 396 (0.54), 366 (0.80), 290 nm (100); log ϵ (λ 366 nm) 4.23; IR (KBr) 1652, 1594, 1506, 1371, 1259, 1234, 1067, 850 cm^{-1}.

Cyclopenta[*cd*]pyren-3(4*H*)-one (3). 1-(Bromoacetyl)pyrene (1.00 g, 3.10 mmol) was dissolved in 850 mL of carbon tetrachloride containing 1 g of lithium carbonate. The solution was irradiated with a medium-pressure TQ 150 mercury arc through a Pyrex

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filter. After irradiation of the solution of 1-(bromoacetyl)pyrene for 100 min, the solvent was removed by evaporation, and the resulting brown solid was adsorbed on a small amount of silica and subsequently chromatographed on a silica column with dichloromethane as the eluent, yielding 0.15 g of 1-(bromoacetyl)pyrene (0.46 mmol, 15%), 0.05 g of 1-acetylpyrene (0.21 mmol, 7%), and 0.29 g of cyclopenta[*cd*]pyren-3(4*H*)-one (120 mmol, 39%). The spectral data are in agreement with those reported by Tintel et al.:[21] ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 3.99 (d, 2 H, H(4,4), J = 1.4 Hz), 8.01 (t, 1 H, H(5), J = 1.4 Hz), 8.09 (t, 1 H, H(7), J = 7.7 Hz), 8.18 (d, 1 H, H(9 or 10), J = 9.2 Hz), 7.24–7.37 (m, 5 H, H(1, 2, 6, 8, and 10 or 9)); MS (150 °C), *m/z* (rel intensity) 242 (100), 214 (71), 213 (66); UV (cyclohexane) λ_{max} (relative ϵ) 400 (0.12), 392 (0.79), 384 (0.17), 371 (0.47), 352 (0.65), 342 (0.41), 337 (0.21), 307 (0.21), 285 (0.75), 275 (0.57), 249 (100); melting point 213–214 °C (lit.[21] mp 214 °C). Determination of a mixed melting point with a sample prepared by the alternate[21] route showed no significant depression.

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Registry No. 1, 129-00-0; 2, 80480-15-5; 3, 69795-70-6; 5, 27208-37-3; $BrCH_2C(O)Br$, 598-21-0; 1-acetylpyrene, 3264-21-9.

Synthesis of (*E*)- and (*Z*)-3-Deuteriophosphoenolpyruvate

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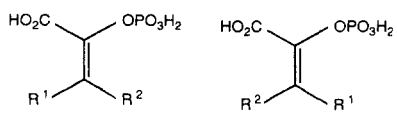
Phosphoenolpyruvate (PEP), a compound with a high phosphate group transfer potential ($\Delta G^{\circ\prime}$ = −14.8 kcal/mol),[1] is a very important biological intermediate.[2] Enzymes that utilize PEP as a substrate may be generally divided into three groups according to the formal chemical reaction catalyzed: (1) reactions that involve the simple hydration of the double bond, (2) reactions that involve an addition of a positively charged atom (H^+ or a carbonyl carbon) to the C-3 position of PEP, which is coupled to the transfer of a phosphate to a nucleophile such as ADP, hexose, or water, and (3) reactions that involve displacement of the phosphate by a nucleophile at the C-2 position of PEP with retention of the double bond.[3] The stereochemical mechanism of only a few of these reactions has been probed due to the limited availability of the PEP stereospecifically labeled at the 3 position with some combination of hydrogen, deuterium, and/or tritium (1 and 2).[4-7] The stereospecifically tritiated analogues are



$R^1 \neq R^2 = {}^1H, {}^2H, \text{or } {}^3H$

R^1 higher priority than R^2

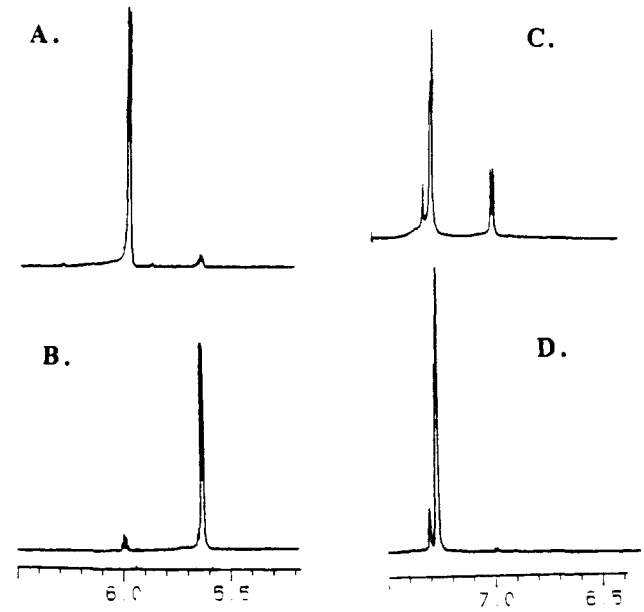


Figure 1. ^{1}H NMR spectra (270 MHz) of (A) ethyl (*Z*)-3-deuterio-2-[(dimethoxyphosphinyl)oxy]propenoate (**5a**); (B) ethyl (*E*)-3-deuterio-2-[(dimethoxyphosphinyl)oxy]propenoate (5b); (C) 72:28 mixture of (*Z*)-**4a** and (*E*)-**4b**; (D) ethyl (*Z*)-3-bromo-2-[(dimethoxyphosphinyl)oxy]propenoate (**4a**). The peak at $\delta \approx$ 7.29 ppm is the residual proton of $CHCl_3$ in the commercial $CDCl_3$.

available by the enzymatic route described by Rose et al.[8,9] and the stereospecifically deuteriated analogues by an elegant synthesis reported by Bartlett.[10] The Bartlett procedure, a rather laborious seven-step synthesis, requires the preparation of special reagents such as 3,5-dinitroperoxybenzoic acid and introduces the hydrogen isotope at a very early stage in the synthesis, making the synthesis of the tritium-labeled analogues quite tedious. Studies recently initiated in our laboratory dealing with the stereochemical mechanism of the formation of UDP-*N*-acetylmuramic acid, 3-deoxy-D-*manno*-octulosonate 8-phosphate, and 3-deoxy-D-*arabino*-heptulosonate 7-phosphate have required PEP stereospecifically labeled in the C-3 position with various hydrogen isotopes. We report here an efficient methodology for the preparation of analogues 1 and 2.

Our synthesis of stereospecifically labeled PEP begins with commercially available ethyl bromopyruvate, which is converted to ethyl 3,3-dibromopyruvate (3) in 80% yield by using *N*-bromosuccinimide in chloroform (see Scheme I). A Perkow-type reaction[11-15] of the dibromo ester with trimethyl phosphite produces a stereoisomeric mixture of ethyl bromophosphoenol pyruvates in which the *Z* to *E*

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