of phosphonopyruvate. Second, the observed retention of configuration at phosphorus rules out the operation of a concerted, pericyclic mechanism. This leaves four intramolecular, stepwise mechanisms (B–E in Scheme III) as being possible for the PEP phosphomutase reaction. Of these four mechanisms, that proceeding via the phosphoenzyme intermediate (pathway E in Scheme III) is the most well precedented.[2] Finally, the similar substrate activity of phosphonopyruvate and thiophosphonopyruvate suggests that, independent of mechanism, nucleophilic attack at the phosphorus is not involved in the rate-limiting step for this rearrangement reaction.[24]

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Supplementary Material Available: Synthetic procedures, spectroscopic data, and NMR spectra of all new compounds reported and X-ray crystallographic data for **15** (37 pages). Ordering information is given on any current masthead page.

(24) Breslow, R.; Katz, I. *J. Am. Chem. Soc.* **1969**, *90*, 7376.

Swertipunicoside. The First Bisxanthone *C*-Glycoside

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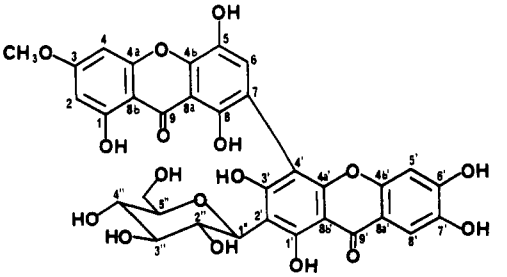
The first bisxanthone *C*-glycoside, swertipunicoside, was isolated from the whole plant of *Swertia punicea* Hemsl. and its structure elucidated through spectroscopic, particularly selective INEPT NMR, analysis as 1,5,8-trihydroxy-3-methoxy-7-(1′,3′,6′,7′-tetrahydroxy-9′-oxo-4′-xanthyl)xanthone 2′-*C*-β-D-glucopyranoside.

Introduction

Seventy-nine of the 170 species of the genus *Swertia* (Gentianaceae) are distributed in China, particularly in the southwestern area.[1] About 20 species of *Swertia* have been used in Chinese traditional medicine for the treatment of hepatic, choleric, and inflammatory diseases.[2,3] *Swertia mileensis* is claimed to be especially efficacious for viral hepatitis.[4] In India, *S. chirata* is used as antimalarial, liver tonic, laxative, febrifuge, stomachic, and bitter tonic.[5,6] The herb of *S. purpurascens* is used in Pakistan as a substitute of *S. chirata*,[7] and in Japan, *S. japonica* is an important bitter stomachic.[8] In previous phytochemical studies, xanthone derivatives,[9–12] flavonoids,[6,13,14] iridoid glycosides,[15–17] and triterpenoids[18,19] have been reported as the main constituents of this genus. Recently, we reported the structure of a new dimeric xanthone, swertiabisxanthone-I, from *S. macrosperma*.[20]

Swertia punicea tastes extremely bitter, possesses the ability to reduce fever and detoxify, and is used in the southwestern part of China for the treatment of hepatogenous jaundice and cholecystitis. No chemical studies have previously been reported for *S. punicea*. Investigation of the whole plant of *S. punicea* has led to the isolation, from the *n*-BuOH fraction of the EtOH extract, of the first member of a new series of natural products, a bisxanthone *C*-glucoside, swertipunicoside (1). The structure was elucidated by a series of NMR experiments, especially the selective INEPT technique.[21–23]



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(1) *Flora of China*; Republicea Popularis Sinicae, Science Press, 1988; p 344.
(2) *A Dictionary of Chinese Traditional Medicines*; Jiangshu New Medical College; Shanghai People's Press: Shanghai, 1977.
(3) Song, W. Z. *Zhong Yao Tong Bao* **1986**, *11*, 643.
(4) The 59th Hospital of People's Liberation Army. *Zhong Cao Yao Tong Xun* **1972**, *5*, 38.
(5) Mandal, S.; Chatterjee, A. *Tetrahedron Lett.* **1987**, *28*, 1309.
(6) Khetwal, K. S.; Verma, D. L. *Indian J. Pharm. Sci.* **1984**, *46*, 25.
(7) Miana, G. A. *Phytochemistry* **1973**, *12*, 728.
(8) Hikino, H.; Kiso, Y.; Kubota, M.; Hattori, M.; Namba, T. *Shoyakugaku Zasshi* **1984**, *38*, 359.
(9) Khetwal, K. S.; Joshi, B.; Bisht, R. S. *Phytochemistry* **1990**, *29*, 1265.
(10) Sun, H. F.; Hu, B. L.; Ding, J. Y.; Fan, S. F. *Zhi Wu Xue Bao* **1991**, *33*, 31.
(11) Nozaka, T.; Morimoto, I.; Watanabe, F.; Okitsu, T. *Shoyakugaku Zasshi* **1984**, *38*, 96.
(12) Ishimaru, K.; Sudo, H.; Satake, M.; Matsunaga, Y.; Hasegawa, Y.; Takemoto, S.; Shimomura, K. *Phytochemistry* **1990**, *29*, 1563.
(13) Hostettman, K.; Jacot-Guillarmod, A. *Helv. Chim. Acta* **1976**, *59*, 1584.
(14) Khetwal, K. S.; Verma, D. L. *Nat. Appl. Sci. Bull.* **1982**, *34*, 337.
(15) Sakai, T.; Nakagawa, Y.; Iwashita, T.; Naoki, H.; Sakan, T. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3477.
(16) Ikeshiro, Y.; Tomita, Y. *Planta Med.* **1984**, *50*, 485; **1985**, *51*, 390.
(17) Hiroe, K.; Shiichi, K.; Nobuji, N. *Chem. Espress* **1988**, *3*, 751.
(18) Prakash, A.; Basumatory, P. C.; Ghosal, S.; Handa, S. S. *Planta Med.* **1982**, *45*, 61.
(19) Zhang, J.; Mao, Q. *Yaoxue Xuebao* **1984**, *19*, 819.
(20) Zhou, H. M.; Liu, Y.-L; Blaskó, G.; Cordell, G. A. *Phytochemistry* **1989**, *28*, 3569.
(21) Cordell, G. A. *Kor. J. Pharmacog.* **1988**, *19*, 153.
(22) Cordell, G. A. *Phytochem. Anal.* **1991**, *2*, 49.

0022-3263/91/1956-7130$02.50/0

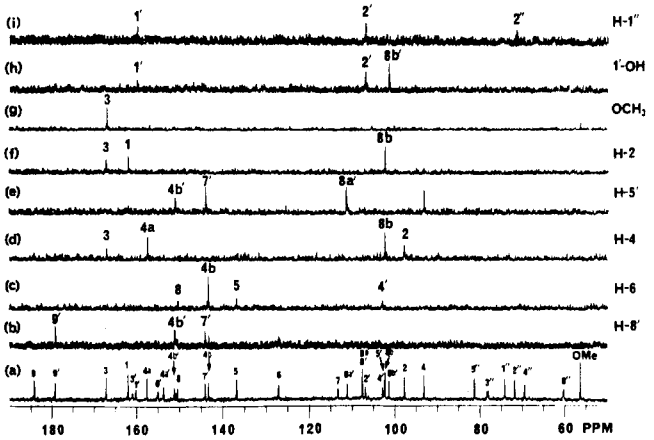

Figure 1. Selective INEPT experiments of swertipunicoside: (a) broad-band decoupled spectrum; (b) irradiation of H-8′, J = 8 Hz; (c) irradiation of H-6, J = 8 Hz; (d) irradiation of H-4, J = 8 Hz; (e) irradiation of H-5′, J = 10 Hz; (f) irradiation of H-2, J = 8 Hz; (g) irradiation of 3-OCH_3, J = 5 Hz; (h) irradiation of 1′-OH, J = 8 Hz; (i) irradiation of H-1″, J = 8 Hz.

Results and Discussion

The proton NMR spectrum of swertipunicoside showed three aromatic proton singlets at δ 6.55 (H-5′), 7.25 (H-6), and 7.37 (H-8′), two meta-coupled doublets at δ 6.45 (H-2, J = 1.4 Hz) and 6.70 (H-4), and a single aromatic methoxy group (δ 3.93). Most of the proton signals of a sugar moiety and some hydroxyl resonances appeared as an unresolved, broad hump in the region δ 3.30–3.90, but a proton doublet at δ 4.84 (J = 9.7 Hz), characteristic for the anomeric proton of C-glycoside, was clearly observed. A relatively downfield signal observed at δ 13.97 indicated that one of the hydroxyl groups was H-bonded. Unambiguous assignment of these proton resonances was made on the basis of the results of HETCOR and selective INEPT experiments.

That swertipunicoside was a bisxanthone C-glycoside was supported by the combined evidence obtained from MS and ^{13}C NMR spectral data. In the ^{13}C NMR spectrum of 1, 33 carbons were observed and the positive ion FABMS indicated a molecular ion peak at m/z 695; the high-resolution mass spectral data confirmed a molecular formula $C_{33}H_{26}O_{17}$. The functionalities included two xanthone moieties, a sugar unit, seven phenolic hydroxy groups, and an aromatic methoxy group. It was recognized that significant challenges to placing the functional groups were posed by the limited number of unsubstituted aromatic carbons. It was also important to distinguish between a C–C and C–O–C structural linkage between the two xanthone nuclei. Through a HETCOR experiment, the protonated ^{13}C resonances were assigned unambiguously. But it was the selective INEPT technique which led to the unequivocal assignment of the quaternary carbon resonances and also permitted the precise placement of the various substituents (Figure 1). Irradiation of H-2 at δ 6.45 (Figure 1f), using a pulse delay corresponding to J = 8 Hz, resulted in the enhancement of the C-8b (δ 102.23), C-1 (δ 161.97), and C-3 (δ 167.11) resonances, and irradiation of H-4 at δ 6.70 (Figure 1d) resulted in the enhancement of C-2 (δ 97.66), C-8b, C-3, and C-4a (δ 157.49). Based on these results, the assignments for C-1, C-2, C-3, C-4, C-4a, and C-8b could be made unambiguously. Furthermore, when the methoxy resonance at δ 3.93 was irradiated, using a pulse delay corresponding to J = 5 Hz, only the resonance at δ 167.11 was significantly enhanced

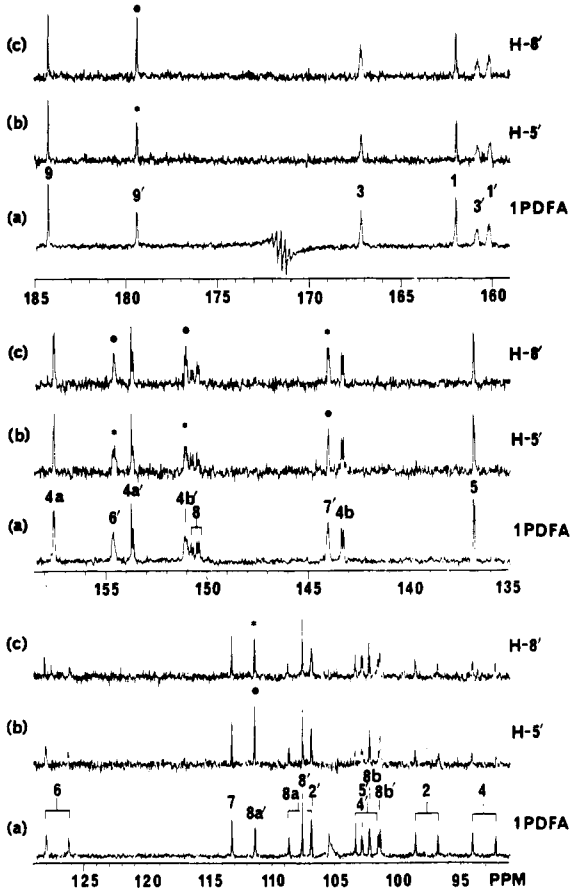

Figure 2. Low-power selective proton decoupling experiments of swertipunicoside: (a) fully coupled ^{13}C spectrum (1PDFA); (b) irradiation of H-5′; (c) irradiation of H-8′. (The mark "●" denotes the carbons which were sharpened due to collapsing of large $^3J_{CH}$ couplings, and the mark "*" denotes the carbons which were simplified due to collapsing of small $^2J_{CH}$ or $^4J_{CH}$ couplings.)

(Figure 1g). Thus, the methoxy group could be affixed to C-3. To further confirm this assignment, a NOESY experiment was performed. Significant correlations were observed between the OCH_3 and H-2 (δ 6.45), and between the OCH_3 and H-4 (δ 6.70).

Selective INEPT experiments showed that irradiation of H-6 at δ 7.25, using a pulse delay corresponding to J = 8 Hz, enhanced four carbon resonances at δ 150.44, 143.26, 136.79, and 102.87 (Figure 1c). The most intense signal at δ 143.27, and a less intense signal at δ 150.44, were assigned to C-4b and C-8, respectively, being three-bond coupled to H-6. When the pulse delay was set longer, corresponding to a J = 5 Hz, it was observed that irradiation of H-6 selectively enhanced only two carbon resonances, at δ 136.79 and 102.87. The more intense signal at δ 136.79 was assigned to C-5 due to two-bond coupling to H-6. Although geminal ^{13}C–^1H couplings in a nonsubstituted benzene are quite small (+1.1 Hz), in substituted aromatic and heteroaromatic systems, due to substitution by different polar substituents, the coupling can vary from −4.9 (for X = F) to +4.2 Hz (for X = $SiMe_3$).[24] This might explain why irradiation of H-6 using a pulse delay corresponding to J = 5 Hz resulted only in the enhancement of the C-5 resonance, and not the C-7 resonance. Examination of the fully coupled ^{13}C NMR spectrum of 1 revealed that the C-5 resonance (δ 136.79) was a small doublet with a coupling constant of 4.1 Hz, and the C-7

(23) Cordell, G. A.; Kinghorn, A. D. *Tetrahedron* **1991**, *47*, 3521.

(24) Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: New York, 1988; pp 512–525.

resonance at δ 113.28 was a sharp singlet (Figure 2a). The multiplicities of these two carbons in the fully coupled carbon spectrum therefore confirmed the assignments. Enhancement of the carbon resonance at δ 102.87 on irradiation of H-6 (Figure 1c) suggested that the linkage between the two xanthone moieties might be 2′–7 or 4′–7 and eliminated a C–O–C linkage between the two xanthone moieties.

On the basis of the results of the selective INEPT experiments, the proton singlets at δ 6.55 and 7.37 could be unambiguously assigned to H-5′ and H-8′, respectively. Irradiation of H-8′ using a pulse delay corresponding to J = 8 Hz resulted in the enhancement of three carbon resonances, at δ 179.25, 151.14, and 144.09 (Figure 1b). When a pulse delay corresponding to J = 5 Hz was used, irradiation of H-8′ enhanced only the resonances at δ 179.25 and 151.14. The carbonyl resonance at δ 179.25 was thus assigned to C-9′ due to three-bond coupling with H-8′. These results permitted a distinction between the carbonyl resonances and allowed the carbonyl resonance at δ 184.19 to be assigned to C-9. Irradiation of H-5′ using a pulse delay corresponding to J = 8 Hz enhanced the carbon resonance at δ 144.09 more significantly than the carbon resonances at δ 151.14 and 111.11. However, when the pulse delay was set shorter, corresponding to J = 10 Hz, two carbon resonances, at δ 144.09 and 111.11, were equally enhanced, and the resonance at δ 151.14 was substantially less enhanced (Figure 1e). Accordingly, it was deduced that the carbon signals at δ 111.11, 144.09, and 151.14 were C-8a′, C-7′, and C-4b′, respectively.

During the course of these NMR experiments, the C–6′ resonance was not identified until the selective proton decoupling experiments were performed (Figure 2). Irradiation of H–8′ employing low decoupling power covering about 20 Hz ($\gamma B_2 \simeq 20$ Hz) not only significantly sharpened the carbon resonances at δ 179.25, 154.97, and 151.14, but also simplified two carbon resonances at δ 144.09 and 111.11 (Figure 2c). On irradiation of H-5′ it was observed that two carbon resonances at δ 144.09 and 111.11 were substantially sharpened, and that the carbon resonances at δ 179.25, 154.97, and 151.14 were simplified significantly (Figure 2b). The carbon signal at δ 154.97 therefore could be assigned to C-6′, and the assignments of C-4b′, C-6′, C-7′, C-8a′, and C-9′ were further confirmed.

In order to determine the position of attachment of the sugar moiety, selective INEPT experiments were performed, irradiating the anomeric proton signal at δ 4.84. When a pulse delay corresponding to J = 5 Hz was used, two carbon resonances, at δ 160.11 and 106.83, were enhanced. Irradiation of the same anomeric proton signal using a pulse delay corresponding to J = 8 Hz, enhanced not only the carbon resonances at δ 160.11 and 106.83, but also the one at δ 71.79 which corresponds to C-2 of the sugar moiety (Figure 1i). The carbon resonance at δ 106.83 which bears the sugar moiety could therefore be tentatively assigned to C–2′ or C-4′ and the resonance at δ 160.11 to C-1′ or C-3′. In other words either the sugar moiety was attached to C-2′ and the upper xanthone unit to C-4′ or vice versa. A distinction between these possibilities could potentially be made through the unambiguous assignment of C-2′ or C-4′ using the hydroxyl-bonded proton at C-1′ and the anomeric proton. Irradiation of the H-bonded OH signal at δ 13.97 using a pulse delay corresponding to J = 5 Hz resulted in the selective enhancement only of the carbon resonance at δ 106.83, thereby confirming that the sugar moiety was attached at C-2′. When the H-bonded OH signal at δ 13.97 was irradiated using a pulse delay corresponding to J = 8 Hz, three carbon resonances, at δ 160.11, 106.83, and 101.31, were enhanced (Figure 1h). The carbon resonance at δ 160.11 must therefore be C-1′, due to $^2J_{CH}$ to the hydroxyl proton. Since irradiation of the anomeric proton also resulted in enhancement of the quaternary resonance at δ 106.83, the location of the sugar moiety at C-4′ could be eliminated. Enhancement of the carbon resonance at δ 101.31 on irradiation of the hydroxyl signal at δ 13.97 permitted unambiguous assignment of this signal to C-8b′. The only remaining position for the lower xanthone unit to be linked to the upper xanthone unit is therefore C-4′. Selective INEPT irradiation of the proton resonance in the upper part of xanthone at δ 7.25 (H-6) allowed the carbon resonance at δ 102.87 to be assigned to C-4′ (Figure 1c) and firmly established the C–C linkage. Accordingly, the seven phenolic hydroxy groups could be assigned to C-1, C-5, C-8, C-1′, C-3′, C-6′, and C-7′. Since the chemical shifts of sugar carbons in swertipunicoside were in good agreement with those described in the literature for a flavonoid *C*-β-D-glucopyranoside,[25] the structure of 1 was deduced to be 1,5,8-trihydroxy-3-methoxy-7-(1′,3′,6′,7′-tetrahydroxy-9′-oxo-4′-xanthyl)-xanthone 2′-*C*-β-D-glucopyranoside.

Experimental Section

Column chromatography was carried out with polyamide (80–100 mesh, Lixian Chemical Co., Hunan Province, China). MPLC was performed on polyamide columns (160–180 mesh). Mass spectra were measured in the positive FAB mode using glycerin and glycerin–NOBA as matrices. For selective INEPT experiments, data sets of 16K covering a spectral width of 10000 Hz were acquired. Proton pulse widths were calibrated by using a sample of HOAc in 10% C_6D_6 (J_{CH} = 6.7 Hz) in a 5-mm NMR tube. The radio frequency field strength for the soft proton pulse was on the order of 20 Hz in these experiments. For J = 10 and 8 Hz, 8000 acquisitions were accumulated in each irradiation and for J = 5 Hz, 20000 acquisitions were accumulated in each irradiation.

Plant Material. The whole plant of *S. punicea* was collected in the northwestern area of Yunnan Province, People's Republic of China, in the fall of 1989. A voucher specimen is deposited in the Herbarium of the Institute of Medicinal Plant Development, Beijing, People's Republic of China.

Isolation of Swertipunicoside. The air-dried whole plant of *S. punicea* (17 kg) was cut into pieces and the extraction initiated with 95% EtOH twice at 50 °C for 2 days, followed by 70% EtOH once for 1 day. The extracts were combined and concentrated in vacuo, and the residue was suspended in H_2O. The suspension was successively extracted with petroleum ether, CH_2Cl_2, EtOAc, and *n*-BuOH, and the extracts were evaporated in vacuo. Half of the *n*-BuOH extract (130 g) was chromatographed on a polyamide column and eluted with H_2O containing an increasing amount of EtOH to yield fractions 1–20 (2 L each) which were collected and monitored by TLC. Fraction 15 was repeatedly subjected to medium-pressure liquid chromatography (MPLC) on polyamide and eluted with $CHCl_3$ containing increasing amounts of MeOH. The fractions were collected according to the UV absorbed bands on the column. The precipitate from fraction 17 eluted with $CHCl_3$–MeOH (8:2) was finally purified on a Sephadex LH-20 column using MeOH as eluent to yield swertipunicoside (1, 150 mg, 0.0009%), yellow powder precipitated from MeOH, mp >360 °C, R_f value 0.15 on polyamide plates developed with MeOH–H_2O (9:1), and 0.42 with CH_3Cl–MeOH (3:1). It gave a positive Molish test and produced an orange color in the HCl–Mg reaction: IR (KBr) ν_{max} 3420 (br, OH), 1640, 1625 (C=O, conj), 1590, 1480 cm^{-1}; UV (MeOH) λ_{max} (log ε) 241 (4.55), 258 (4.56), 280 (4.42), 319 (4.36), and 368 nm (4.10); (MeOH + NaOMe) 245, 283, 338 and 392 nm; (MeOH + NaOAc) 244 sh, 284, 336 and 388 nm; (MeOH + NaOAc + H_3BO_3) 257 sh, 283, 330, and 420 nm; (MeOH + $AlCl_3$) 238, 271, 282 sh, 334, and 386 nm; (MeOH + $AlCl_3$ + HCl) 238, 263, 284, 334, and 386 nm; ^{1}H

(25) Harborne, J. B.; Mabry, T. J. *The Flavonoids: Advances in Research*; Chapman & Hall, London, 1982; pp 48–51.

NMR (DMSO-d_6, 361.1 MHz) δ 13.97 (s, OH-1'), 7.37 (s, H-8'), 7.25 (s, H-6), 6.70 (d, J = 1.4 Hz, H-4), 6.55 (s, H-5'), 6.45 (d, J = 1.4 Hz, H-2), 4.84 (d, J = 9.7 Hz, H-1''), 3.93 (s, OCH_3); ^{13}C NMR (DMSO-d_6, 90.8 MHz) δ 184.19 (s, C-9), 179.25 (s, C-9'), 167.11 (s, C-3), 161.97 (s, C-1), 160.93 (s, C-3'), 160.11 (s, C-1'), 157.49 (s, C-4a), 154.97 (s, C-6'), 153.67 (s, C-4a'), 151.14 (s, C-4b'), 150.44 (s, C-8), 144.09 (s, C-7'), 143.27 (s, C-4b), 136.79 (s, C-5), 127.10 (d, C-6), 113.28 (s, C-7), 111.11 (s, C-8a'), 107.57 (s/d, C-8a, C-8'), 106.83 (s, C-2'), 102.87 (s, C-4'), 102.34 (d, C-5'), 102.23 (s, C-8b), 101.31 (s, C-8b'), 97.66 (d, C-2), 93.08 (d, C-4), 81.24 (d, C-5''), 77.98 (d, C-3''), 74.16 (d, C-1''), 71.79 (d, C-2''), 69.45 (d, C-4''), 60.20 (t, C-6''), 56.37 (q, OCH_3); HRMS (positive FAB) m/z (rel int) found [M^+ + H] 695.1248, calcd for $C_{33}H_{27}O_{17}$ [M^+ + H] 695.1251; 695 [M^+ + 1] (100), 677 (9), 631 (8), 617 (19), 603 (19), 589 (7), 575 (13), 571 (7), 569 (13), 561 (24), 559 (14), 557 (17), 545 (14).

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Synthesis and Evaluation of Glucose-ADP Hybrids as Inhibitors of Hexokinase

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Glucose-ADP hybrids, in which carboxamide (**4**) and linear acetylene (**5**) and allene (**6**) functional groups link the two moieties, were designed as potential multisubstrate analogue inhibitors for hexokinase. The diastereomeric aldehydes **15** and **28** were key intermediates in the synthesis of these compounds. Reduction of **15**, amination, and acylation with (diethylphosphono)acetic acid provided the amide phosphonate **20**. Reduction of **15**, three-carbon extension to the propargylic bromide **25**, and Arbuzov displacement gave the acetylenic phosphonate **26**. Addition of ethynylmagnesium bromide to aldehyde **28**, separation of the diastereomers, and Mark rearrangement afforded the allenic phosphonates **31R** and **31S**. Cleavage of the phosphonate esters (trimethylsilyl bromide) and acetal hydrolysis (90% aqueous trifluoroacetic acid) furnished the corresponding deprotected phosphonic acids, which were coupled with adenosine 5'-monophosphate through activation with carbonyl di(imidazole). Inhibition of yeast hexokinase by carboxamide **4** (K_i = 0.2 mM) and acetylene **5** (K_i = 2.5 mM) is competitive with glucose and noncompetitive with ATP; the *R*-allene **6R** (IC_{50} = 1.7 mM) and *S*-allene **6S** (IC_{50} = 10 mM) are also weak inhibitors. It was concluded that these compounds are not functioning as multisubstrate analogues. The β,γ-methylene-γ-methylthio analogue of ATP (**7**) was also synthesized. This compound in combination with glucose, as well as γ-thio-ATP (**9**) in combination with 6-deoxy-6-iodoglucose (**8**), were investigated for potential enzyme-induced, covalent coupling. No evidence for such coupling was observed.

Introduction

Kinases are a ubiquitous and important class of enzymes that transfer the terminal phosphate group from nucleoside triphosphates, usually ATP, to a nucleophilic acceptor. These enzymes play a variety of roles in primary metabolism in the regulation of enzyme activity, in signal transduction, and in the modulation of cellular growth and differentiation.[1] In spite of their importance, no general strategies have been devised for inhibiting kinases. For other broad enzyme classes, such as peptidases and transaminases the synthesis of transition state analogues[2] or suicide inhibitors[3] are effective strategies. These approaches have proven to be elusive for the kinases, however, since an effective mimic has yet to be found for the γ-phosphate moiety in the pentavalent, trigonal bipyramidal geometry that it adopts in the transition state (e.g., **1**) and since no way has been discovered to use such a reaction to trigger a process that could lead to irreversible inactivation of the enzyme.

The ternary nature of the enzyme-substrate complex is a striking feature of the kinase reaction, and it suggests that the design of "multisubstrate analogues"[4] could be an effective approach to the design of inhibitors in which the phosphate acceptor is a small molecule. Indeed, P^1,P^5-di(adenosine-5')pentaphosphate (Ap_5A, **2**), an inhibitor of adenylate kinase, was one of the first multisubstrate analogues to be characterized.[5] Adenylate kinase is strongly inhibited by Ap_5A ($K_i = 2.5 \times 10^{-9}$ M) but not by the homologues with fewer phosphoryl groups in the polyphosphate bridge (Ap_4A: $K_i = 2.4 \times 10^{-5}$ M).[6] The extra phosphate group in Ap_5A is believed to compensate for the

(1) Kanoh, H.; Yamada, K.; Sakane, F. *Trends Biochem. Sci.* **1990**, *15*, 47-50. Taylor, S. S.; Buechler, J. A.; Yonemoto, W. *Annu. Rev. Biochem.* **1990**, *59*, 971-1005. Yarden, Y.; Ullrich, A. *Ibid.* **1988**, *57*, 443-478. Hunter, T.; Cooper, J. A. In *The Enzymes*; Boyer, P. D., Krebs, E. G., Eds.; Academic Press: Orlando, FL, 1986; Vol. 17, pp 191-246. Eventoff, W.; Rossmann, M. G. *CRC Crit. Rev. Biochem.* **1975**, *3*, 111-140.

(2) (a) Wolfenden, R. *Acc. Chem. Res.* **1972**, *5*, 10-18. (b) Stark, G. R.; Bartlett, P. A. *Pharmacol. Ther.* **1983**, *23*, 45-78. (c) Schray, K.; Klinman, J. P. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 641-648. (d) Frick, L.; Wolfenden, R. In *Design of Enzyme Inhibitors as Drugs*; Sandler, M., Smith, H. J., Eds.; Oxford University Press: New York, 1989; pp 19-48.

(3) Walsh, C. *Tetrahedron* **1980**, *38*, 871-909. Silverman, R. B. *Mechanism Based Enzyme Inactivation*; CRC Press: Boca Raton, FL, 1988; Vols. 1 and 2.

(4) (a) Jencks, W. P. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1975**, *43*, 219-410. (b) Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1678-1683.

(5) Lienhard, G. E.; Secemski, I. I. *J. Biol. Chem.* **1973**, *248*, 1121-1123.

(6) Purich, D. L.; Fromm, H. J. *Biochem. Biophys. Acta* **1972**, *276*, 563-567.

0022-3263/91/1956-7133$02.50/0