Tetrahedron Vol. 43, No. 22, pp. 5261 to 5274, 1987
Printed in Great Britain.

0040-4020/87 $3.00+.00

PTAQUILOSIDE, A POTENT CARCINOGEN ISOLATED FROM BRACKEN FERN PTERIDIUM AQUILINUM VAR. LATIUSCULUM: STRUCTURE ELUCIDATION BASED ON CHEMICAL AND SPECTRAL EVIDENCE, AND REACTIONS WITH AMINO ACIDS, NUCLEOSIDES, AND NUCLEOTIDES

MAKOTO OJIKA, KAZUMASA WAKAMATSU, HARUKI NIWA,
AND KIYOYUKI YAMADA*

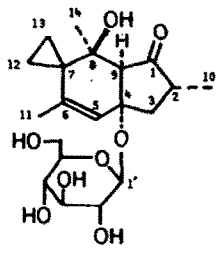
Department of Chemistry, Faculty of Science, Nagoya University,
Chikusa, Nagoya 464 Japan

(*Received in Japan* 4 *August* 1987)

Abstract - The structure of ptaquiloside (**1**), a potent carcinogenic compound isolated from bracken fern, Pteridium aquilinum var. latiusculum has been elucidated on the basis of chemical and spectral evidence. The dienone **3** generated from **1** under alkaline conditions was shown to be a strong alkylating agent. For the purpose of obtaining the preliminary information on chemical modification of biopolymers such as proteins and DNA with the dienone **3**, reactions of **3** with amino acids, nucleosides, and nucleotides have been carried out and the alkylated products have been characterized.

Bracken fern, Pteridium aquilinum var. latiusculum is widely distributed throughout the world and used in Japan as a food. The lethal properties of bracken fern to cattles were first reported in the late 19th century.[1] Cattles that consumed bracken fern exhibited the syndrome known as "cattle bracken poisoning," the major features of which are the generalized hemorrhage, anorexia, extensive intestinal damage, ulceration, and pyrexia.[1] Extensive studies on the cattle bracken poisoning factor have been carried out, but the factor has not as yet been isolated.[1]

As to the carcinogenic properties of bracken fern the earliest finding was reported in 1960 by Rosenberger and Heeschen.[1,2] In 1965 Evans and Mason unambiguously proved the carcinogenicity of bracken fern: rats which consumed bracken fern developed multiple intestinal adenocarcinomas.[3] Subsequently, this result was confirmed by other workers.[4,5] In search for the carcinogenic principle of bracken fern, a great deal of chemical work on bracken fern has so far been performed.[1,6] Although a large number of known and new compounds have been isolated from bracken fern, none of them were carcinogenic.[1,6] Thus, the isolation of the carcinogen from bracken fern has been a long standing problem. Among the several factors which have made it extremely difficult to isolate the carcinogen from bracken fern, the following two factors are notable: difficulty of applying the various short-term bioassay systems and the instability of the carcinogen.[2,7]



1

Taking these factors into consideration, we have conducted fractionation of the aqueous extract of bracken fern by means of the assay based on carcinogenicity to rats,[8,9] isolated a new, unstable norsesquiterpene glucoside named ptaquiloside (**1**),[8,9] determined the structure,[9-11] and proved that ptaquiloside (**1**) is the carcinogen of bracken fern[8,12] and the causative principle of cattle bracken poisoning[13] as well. Since the original method[8,9] for isolating ptaquiloside (**1**) was complicated, we have devised an efficient and convenient method for the isolation of ptaquiloside (**1**) in a yield five times greater than the original procedure.[14]

In this paper we wish to describe the properties of ptaquiloside (**1**) and the details of structural elucidation of **1** based on chemical and spectral evidence,[15] and also report the reactivities toward amino acids, nucleosides, and nucleotides, the studies of which would provide us with the basic information on the chemical modification of biopolymers with ptaquiloside (**1**) as an alkylating agent.

Properties and Structure of Ptaquiloside (1)

Originally, the extraction procedure of ptaquiloside (**1**) guided by the carcinogenic activity toward rats was reported[8] and later the modified and efficient method for isolation of **1** was developed.[14] Although ptaquiloside (**1**) can practically be obtained by the modified method, the details of the chemical aspect of the original procedure for extraction of **1** are described in the experimental section.

Ptaquiloside (**1**) was obtained as a colorless, amorphous, and hygroscopic powder. Ptaquiloside (**1**) is readily soluble in water and fairly soluble in ethyl acetate (ca. 100 mg/ml EtOAc), and its partition between water and ethyl acetate is about 6 (H_2O phase)/1 (EtOAc phase) by weight. Ptaquiloside (**1**) in a dry atmosphere is stable at room temperature at least for several days and at low temperature (-20 - 0 °C) for more than six months, but is gradually decomposed, when it is kept in aqueous or alcoholic solution at room temperature. The molecular formula and the spectral properties of ptaquiloside (**1**) are as follows.

$C_{20}H_{30}O_8$; $[\alpha]_D^{22}$ -188° (c 1.00, MeOH);
IR (KBr) 3400 (broad), 1724, 1640 (weak) cm^{-1};
^1H NMR, Table 2; ^{13}C NMR, Table 3;
FABMS m/z 421 $(M + Na)^+$.

Ptaquiloside (**1**) is unstable under both acidic and basic conditions at room temperature, and underwent aromatization with liberation of D-(+)-glucose to give 1-indanone derivatives such as pterosin B (**4**)[16,17] and pterosin O (**5**),[18] depending on the solvent used. The half-life of ptaquiloside (**1**) is listed in Table 1. Acetylation of **1** with acetic anhydride and pyridine afforded crystalline ptaquiloside tetraacetate (**2**), $C_{28}H_{38}O_{12}$, mp 173-174 °C (dec.). In the alkaline aqueous solution (pH 8-11) at room temperature ptaquiloside (**1**) was transformed with concomitant liberation of D-(+)-glucose into an unstable conjugated dienone **3**, $C_{14}H_{18}O_2$ [UV (CH_3CN) 321 (ε 9800), 213 nm (8040); IR (CCl_4) 1667, 1570 cm^{-1}] as a colorless oil. The dienone **3** was extremely unstable in the weakly acidic aqueous solution at room temperature and immediately converted to pterosin B (**4**), while **3** was stable in the alkaline solution at room temperature at least for 1 hour.

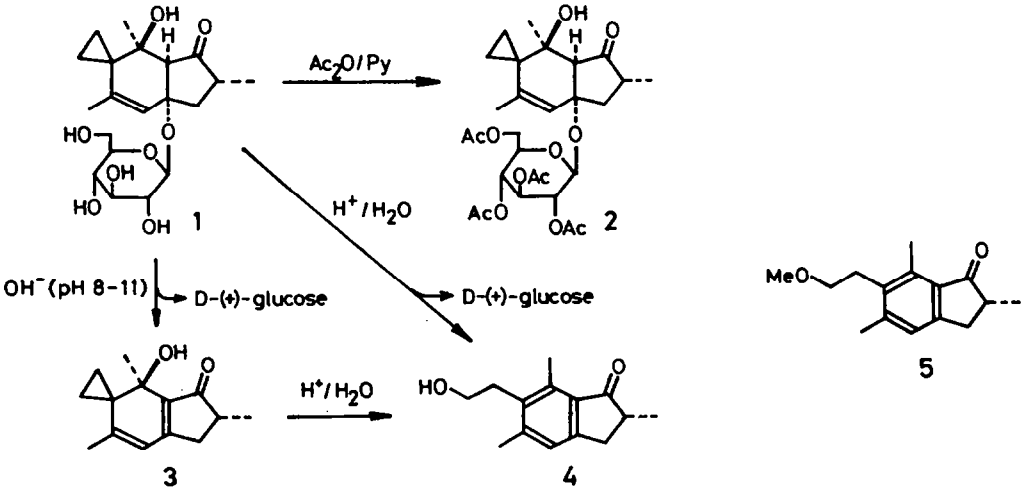


Table 1. Half-life of Ptaquiloside (1)

conditions	pH	half-life
5 mM H_2SO_4 25 °C	2.0	2.9 h
10 mM Na_2CO_3 buffer 25 °C	9.0	40 min
	10.0	6.3 min
	11.0	69 sec

Table 2. ^1H NMR Spectral Data (CD_3OD)[a]

proton	1[b]	2[c]	3[c]
2	2.23 ddq (12.5, 8.0, 6.9)	2.23 ddq (12.2, 8.2, 7.0)	2.47 ddq (6.7, 2.4, 7.6)
3a	1.93 t (12.5)	1.93 t (12.2)	2.18 dd (18.6, 2.4)
3b	2.49 dd (12.5, 8.0)	2.42 dd (12.2, 8.2)	2.85 dd (18.6, 6.7)
5	5.76 dq (1.3, 1.0)	5.73 quint (1.5)	6.11 q (1.2)
9	2.64 d (1.3)	2.53 d (1.5)	-
10	1.07 d (6.9)	1.07 d (7.0)	1.15 d (7.6)
11	1.53 d (1.0)	1.55 d (1.5)	1.74 d (1.2)
12a	0.48 m*	0.51 m*	0.62 ddd (9.8, 7.0, 4.3)*
12b	0.86 m*	0.87 m*	1.06 ddd (9.8, 6.7, 4.9)*
13a	0.69 m*	0.71 m*	0.92 ddd (9.8, 7.0, 4.9)*
13b	0.86 m*	0.87 m*	1.40 ddd (9.8, 6.7, 4.3)*
14	1.29 s	1.15 s	1.24 s
1'	4.60 d (7.6)	5.07 d (7.9)	-
2'	3.20 dd (8.9, 7.6)	4.90 dd (9.7, 7.9)	-
3'	†	5.28 t (9.7)	-
4'	3.36 t (9.0)	4.98 dd (10.1, 9.7)	-
5'	†	3.94 ddd (10.1, 5.5, 2.5)	-
6'a	3.66 dd (11.9, 5.6)	4.17 dd (12.2, 5.5)	-
6'b	3.90 dd (11.9, 1.3)	4.25 dd (12.2, 2.5)	-
Ac	-	1.95, 1.96, 2.02, 2.07	-

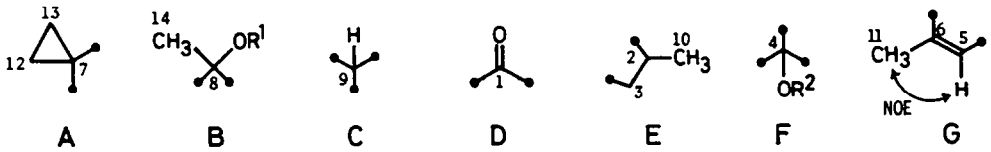
a) Chemical shifts are in ppm relative to TMS. Coupling constants in Hz are in parentheses.
b) Observed at 270 MHz.
c) Observed at 400 MHz.
* These values are interchangeable within a vertical column.
† These signals could not be observed by overlapping with a solvent signal.

Table 3. ^{13}C NMR Spectral Data (67.8 MHz)[a]

carbon	1 (CD_3OD)	2[b] (C_6D_6)
1	224.9 s	221.1 s
2	41.1 d (120.4)	44.0 d (121.5)
3	46.1 t (130.8)	44.5 t (133.9)
4	83.0 s	81.4 s
5	124.2 d (161.1)	120.7 d (156.8)
6	145.3 s	145.2 s
7	31.0 s	29.8 s
8	72.9 s	70.1 s
9	63.5 d (129.2)	61.4 d (126.7)
10	14.5 q (125.5)	13.4 q (127.9)
11	20.3 q (125.0)	19.5 q (126.4)
12	6.8 t (159.5)	5.8 t (161.1)
13	11.5 t (159.5)	10.8 t (160.8)
14	27.9 q (126.0)	26.9 q (127.2)
1'	100.2 d (159.9)	96.1 d (160.9)
2'	76.2 d (143.1)	71.8 d (142.8)*
3'	78.7 d (141.2)*	72.1 d (154.1)*
4'	72.9 d (143.5)	69.1 d (150.1)
5'	79.2 d (141.5)*	73.3 d (151.4)
6'	63.9 t (143.6)	62.3 t (148.3)

a) Chemical shifts are ppm relative to TMS. The values in parentheses are $^1J_{C-H}$.
b) Signals due to the acetate groups: 168.6, 169.1 169.8, 169.9, 20.1, 20.2, 20.3 (2C).
* These values are interchangeable within a vertical column.

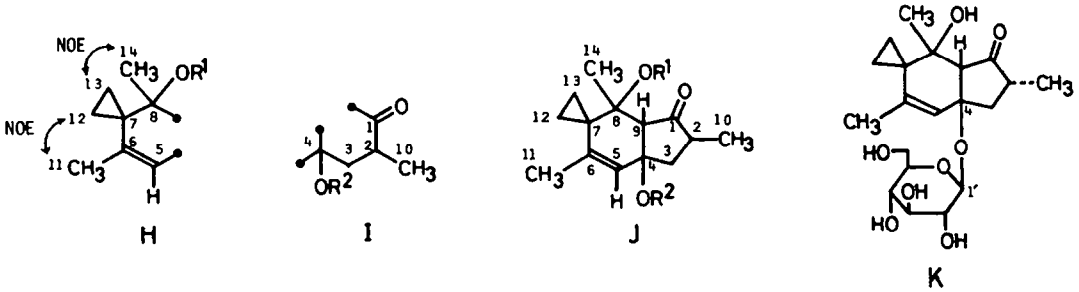
The NMR spectral data of ptaquiloside (**1**) and the derivatives are shown in Table 2 and 3. The ^{1}H NMR and ^{1}H NMDR spectra of ptaquiloside (**1**) together with the proton noise-decoupled and off-resonance ^{13}C NMR spectra disclosed the presence of the partial structures, **A** - **G**.



R^1, R^2 = -H, -$C_6H_{11}O_5$ (D-(+)-glucose)

In the ^{13}C NMR spectrum of **1**, three signals at δ_C 31.0 (s, C-7), 6.8 (t, J = 159.5 Hz, C-12), and 11.5 (t, J = 159.5 Hz, C-13) suggested the presence of the 1,1-disubstituted cyclopropane ring (**A**) in **1**, which was further supported by the ^{1}H NMR spectrum of **1** [δ_H 0.48 (1H, m, H-12a), 0.69 (1H, m, H-13a), and 0.86 (2H, m, H-12b and H-13b)]. The cis relationship between the methyl group [δ_H 1.53 (3H, d, J = 1.0 Hz, H-11)] and the vinyl hydrogen [δ_H 5.76 (1H, dq, J = 1.3 and 1.0 Hz, H-5)] in **G** was substantiated by the differential NOE experiment of **1**. The spin decoupling experiments of H-2 [δ_H 2.23 (1H, ddq, J = 12.5, 8.0, and 6.9 Hz)] and H-3 [δ_H 1.93 (1H, t, J = 12.5 Hz) and 2.49 (1H, dd, J = 12.5 and 8.0 Hz)] in **E** revealed that H-2 was coupled solely to H-3 and H-10 and that H-3 was coupled only to H-2, suggesting that two terminal carbons (C-2 and C-3) in **E** were connected to quaternary carbons, respectively.

Since the partial structures, **A** - **G** contain all carbon atoms in ptaquiloside (**1**), the remaining problem is the correlation of these partial structures, which could be achieved by long range selective proton decoupling experiments (LSPD).[19] In the proton coupled ^{13}C NMR spectrum of **1** under the gated decoupling experiment, all quaternary carbon signals at δ_C 31.0 (C-7 in **A**), 72.9 (C-8 in **B**), 224.9 (C-1 in **D**), 83.0 (C-4 in **F**), and 145.3 (C-6 in **G**) appeared as the broad singlets with fine splittings arising from two and/or three bond C-H couplings ($^2J_{C-H}$ and/or $^3J_{C-H}$). (a) LSPD irradiating H-14 (δ_H 1.29) in **B** collapsed the C-7 (δ_C 31.0) and C-8 (δ_C 72.9) carbon signals to the better defined broad singlets, revealing that the protons (H-14) of the methyl group in **B** are three bonds away from the C-7 quaternary carbon in **A**: the cyclopropane ring in **A** is therefore connected to the C-8 carbon in **B**. (b) The splitting pattern of the C-7 carbon signal in **A** was also simplified upon irradiation of H-11 (δ_H 1.53) in **G**, indicating that the C-6 carbon is bonded directly to the C-7 carbon. These findings, (a) and (b), made it possible to correlate three partial structures, **A**, **B**, and **G**, leading to a new, extended partial structure (**H**), which was supported by the observation of the NOE between H-14 in **B** and H-13a in **A**, and between H-11 in **G** and two proton signals of the cyclopropane ring [H-12a (or H-12b) and H-13b] in **A**. (c) LSPD irradiating H-10 (δ_H 1.07) in **E** collapsed only the C-1 carbonyl carbon signal in **D** to a simple singlet, indicating that the C-2 carbon in **E** is connected to the C-1 carbon in **D**. (d) Since the C-2 and C-3 carbons in **E** were deduced to be connected to quaternary carbons as described above, the remaining quaternary carbon C-4 (δ_C 83.0) in **F** is necessarily bonded



to the C-3 carbon in **E**. On the basis of these findings, (c) and (d), correlation of three partial structures, **D**, **E**, and **F**, became possible to afford a new, extended partial structure (**I**). (e) LSPD of H-9 (δ_H 2.64) in **C** dramatically simplified each of three quaternary carbon signals (C-1, C-4, and C-8), revealing that the C-9 carbon in **C** is linked directly to C-8 in **H** and also C-1 and C-4 in **I** to give the planar structure with the illudane skeleton (**J**) for ptaquiloside (**1**). The planar structure (**J**) for **1** was chemically consistent with the formation of pterosin B (**4**) from **1** as described above.

The site and stereochemistry of the glycosidic linkage in ptaquiloside (**1**) were established as follows. The β-configuration of the glycosidic linkage in **1** was determined by the coupling constant of the anomeric proton signal H-1' [δ_H 4.60 (1H, d, J = 7.6 Hz)] and the C-H coupling constant of the anomeric carbon signal C-1' (δ_C 100.2, J_{C-H} =159.9 Hz). The location of the glycosidic linkage was disclosed by LSPD of the anomeric proton signal H-1' in ptaquiloside (**1**): irradiation of H-1' in **1** eliminated a long range coupling from the C-4 carbon signal, revealing that D-(+)-glucose is bonded to the hydroxy group at C-4. The confirmative evidence for the location of the glycosidic linkage is the formation of the conjugated dienone **3** with simultaneous liberation of D-(+)-glucose on treatment of **1** with aqueous base.

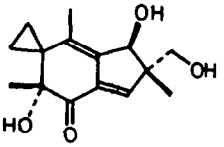
The absolute stereochemistry at C-2 bearing the secondary methyl group in the structure (**J**) was shown to be R, because, as described, ptaquiloside (**1**) was led to (-)-pterosin B (**4**), the absolute stereochemistry of which is known.[17]

Thus, on the basis of the detailed spectral analysis and chemical reactions of ptaquiloside (**1**) and the derivatives described above, the structure of ptaquiloside was established to be the formula (**K**).

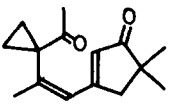
We have determined the whole stereostructure including absolute stereochemistry of ptaquiloside to be represented by **1** in terms of X-ray crystallographic analysis of ptaquiloside tetraacetate (**2**).[10,11]

In 1983 van der Hoeven and coworkers isolated a new compound, aquilide A from bracken fern Pteridium aquilinum (L.) Kuhn and reported the planar structure, which is the same as that of ptaquiloside (**1**).[20] Aquilide A seems to be identical with ptaquiloside (**1**) by comparison of the NMR spectral data of both compounds.

Ptaquiloside (**1**) is a glucoside of norsesquiterpene with the illudane skeleton. Natural occurrence of the sesquiterpenes of this type is rare:[21,22] illudin S (**6**)[23-25] isolated as a toxic compound from the bioluminescent mushroom is worthy of note as a representative example of the illudane type sesquiterpenes so far known. It is notable that while ptaquiloside (**1**) reveals potent carcinogenicity, illudin S (**6**) shows antitumor activity. Further it should be noted that ptaquiloside (**1**) is structurally related to hypacrone (**7**),[26] an acrid principle of a fern Hypolepis punctata. Previously, many aromatic sesquiterpenes having the 1-indanone skeleton named pterosins [e.g., pterosin B (**4**)] and pterosides have been isolated as characteristic constituents of bracken fern.[6,16] Biogenetically ptaquiloside (**1**) can be regarded as a precursor of pterosins and pterosides.



6

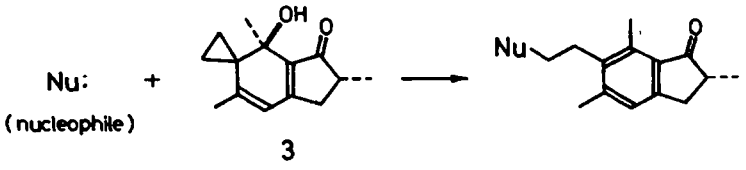


7

The instability of ptaquiloside (**1**) is ascribed to the ready aromatization accompanied by liberation of D-(+)-glucose. As described above, the pathway to aromatic compounds from **1** under alkaline conditions is the rapid conversion of **1** with elimination of D-(+)-glucose into the unstable dienone **3**, which is then slowly transformed into aromatic compounds. Under neutral conditions the pathway to aromatic compounds from **1** is essentially the same as that under alkaline conditions: **1** is gradually converted to **3**, which is transformed into aromatic compounds faster than in the case under alkaline conditions. The pathway leading to aromatic compounds from **1** under acidic conditions is uncertain. Further studies are needed for the mechanism of aromatization of **1** under acidic conditions.

Reactions of Ptaquiloside (1) with Amino Acids, Nucleosides, and Nucleotides

Chemical properties of ptaquiloside (**1**) and its derivative dienone **3** described above show that they act as alkylating agents toward a variety of nucleophiles. The covalent binding of carcinogenic alkylating agents with DNA is regarded as an important event in the mechanism of action of these agents, and these chemical modifications of DNA are generally thought to be the initial step of chemical carcinogenesis.[27] Carcinogens can be divided into those that react directly with macromolecules such as DNA and proteins without enzymatic activation, and those that require metabolic activation.[27,28] Chemical properties of ptaquiloside (**1**) indicate that **1** is classified as a directly acting carcinogen and that the dienone **3** generated from **1** under alkaline and neutral conditions can be regarded as the active form of **1**. The cyclopropane ring in **3** is highly reactive as an electrophile, which is conjugated with the keto group and constitutes a cyclopropylcarbinol system as well: thus the cyclopropane ring in **3** can react with a variety of nucleophiles (e.g., water, alcohols, amines, etc.) quite readily.



3

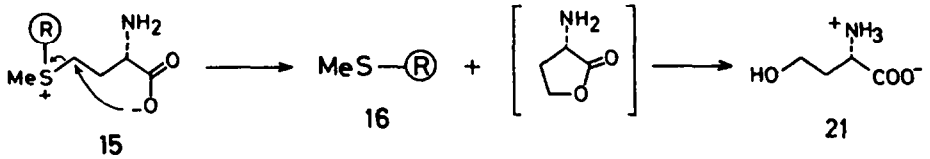
We have investigated the reactivities of dienone **3**, the active form of ptaquiloside (**1**) toward amino acids, nucleosides, and nucleotides in order to obtain preliminary and basic information on the chemical modifications of DNA and proteins with **3**. Further, in view of the fact that ptaquiloside (**1**) is the active principle of cattle bracken poisoning, the studies on the reactivities of **3** with amino acids are significant from the standpoint of searching for the effective compounds for detoxication of **1**, which is the important problem in the veterinary science field.

Reaction of Dienone 3 with Amino Acids

Four amino acids were investigated for the reaction with **3**: as a typical amino acid alanine (**8**) was selected and three sulfur-containing amino acids, cysteine (**9**), methionine (**10**), and glutathione (**11**) were used.

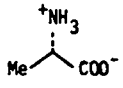
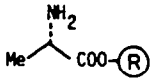
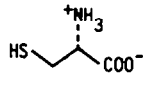
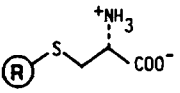
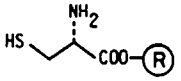
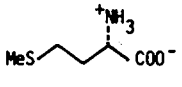
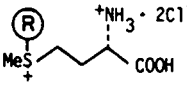
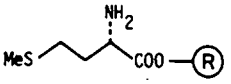
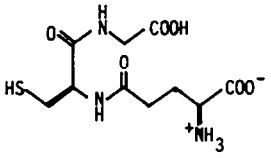
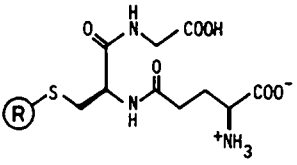
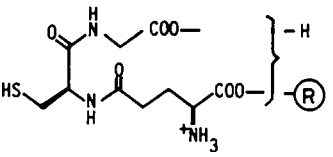
Dienone **3** was reacted with amino acids under the following conditions: 0.05 M dienone **3** and 0.10 M amino acid in aqueous acetone, pH 7.5, 37 °C, 2 hours (aqueous THF was used as solvent in the case of **9**). After separation of the mixture of the products, each product was purified and characterized by spectroscopic and chemical means as described in the experimental section, and the results are summarized in Table 4.

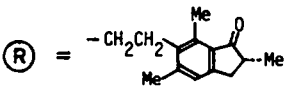
Since **3** reacted with water used as solvent to a large extent, pterosin B (**4**) was always formed as the major product in each case. The yield of the product was based on dienone **3**. The carboxylate group in the amino acids was alkylated with **3** to a small extent (1-4% in **8**, **9**, **10**, and **11**). Alkylation of the thiol group in cysteine (**9**) and glutathione (**11**) with **3** occurred to a considerable extent (21% in **9** and 8.1% in **11**). Even a sulfide group was found to react with **3**. Alkylation of methionine (**10**) with **3** took place to give the corresponding sulfonium salt **15** in 23% yield. A small amount of the S-alkylated product **16** (0.8%) was detected, which resulted from the sulfonium salt **15** by the intramolecular displacement reaction. Under alkaline conditions (pH 9.2) formation of **16** was



15 **16** **21**

Table 4. Alkylation of Amino Acids with Dienone **3**

amino acid	products (yield)		
 **8**	 **12** (3.5%)		
 **9**	 **13** (21%)	 **14** (1.4%)	
 **10**	 **15** (23%)	 **16** (0.8%)	 **17** (2.9%)
 **11**	 **18** (8.1% as the ammonium salt)	 two isomers **19** (2.4%), **20** (2.2%)	



increased (12%), and homoserine (**21**) was isolated.

These results indicate that alkylation of the thiol and sulfide groups in the amino acids with **3** takes place to a considerable extent. Since the 1-indanone derivatives such as pterosin E (**4**) are known to show no carcinogenicity,[6] the sulfur-containing amino acids can potentially be used as detoxicating agents for ptaquiloside (**1**).

Reaction of Dienone 3 with Nucleosides

Alkylation of four nucleosides, adenosine (**22**), guanosine (**23**), uridine (**24**), and cytidine (**25**) with **3** was examined. The alkylation conditions for these nucleosides except for guanosine (**23**) are as follows: 0.05 M dienone **3** and 0.10 M nucleoside in aqueous acetone, pH 7.5, 37 °C, 2 hours. Owing to the low solubility of guanosine (**23**) to the solvent system, the alkylation experiment was executed in an aqueous acetone solution of 0.0125 M each of dienone **3** and guanosine (**23**) (pH 7.5, 37 °C, 2 hours). The adducts were separated and purified, and their structures were established by the spectroscopic method: the site of alkylation in the purine and pyrimidine bases of the nucleosides was determined using the difference UV spectra in acidic, neutral, and basic solutions by comparison with the UV spectra of the known methylated and ethylated nucleosides. The difference UV spectra of the alkylated nucleosides were obtained by subtracting the UV spectrum of pterosin B (**4**) from those of the alkylated nucleosides: each of the difference UV spectra thus obtained should correspond to that of a specifically substituted nucleoside (Table 5). The results are listed in Table 6. As in the case of amino acids described above, pterosin B (**4**) was overwhelmingly formed in each case. The yield of the adduct was based on dienone **3**.

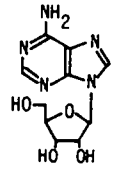
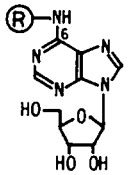
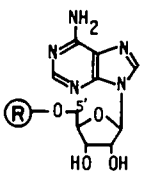
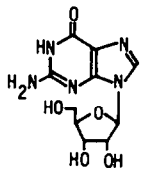
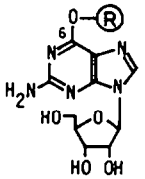
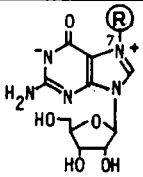
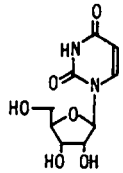
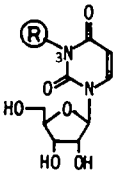
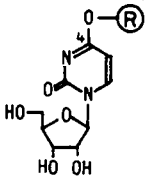
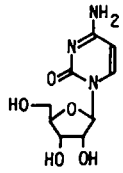
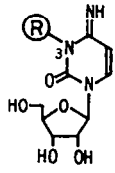
In the alkylation of adenosine (**22**) there were isolated two products, **26** (0.3%) and **27** (0.4%): the former **26** was the product alkylated at the N^6 position of adenine and the latter **27** was shown to be

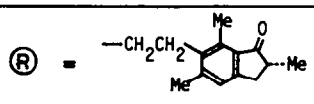
Table 5. Difference UV Spectra of Dienone-nucleoside Adducts and UV Spectra of Methylated and Ethylated Nucleosides

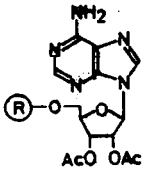
nucleoside	alkylated nucleoside	pH 1[a] λ max	pH 1[a] λ min	pH 7[b] λ max	pH 7[b] λ min	pH 13[c] λ max	pH 13[c] λ min
	26	266	237	272	240	272	240
adenosine	N^6-Et-adenosine	264	239	268	242	268	243
	27	255	232	257	235	257	232
	adenosine	257	231	259	227	260	233
	28	242, 286	235, 261	247, 277	235, 261	251, 275	235, 261
guanosine	O^6-Et-guanosine	244, 286	233, 260	248, 277	229, 261	247, 278	233, 261
	29	257, 282(sh)	243	258, 280(sh)	234		
	N-7-Et-guanosine	257, 277(sh)	244	257, 277(sh)	235		
	30	262	236	262	236	263	237
uridine	N-3-Me-uridine	262	238	262	240	263	241
	31	273	241	273	241	274	241
	O^4-Me-uridine	271	235	274	239	274	239
	32	281	242	280	243	268	245
cytidine	N-3-Et-cytidine	280	247	279	246	267	248

a) HCl/H_2O-MeOH (8:2). b) NH_4OAc/H_2O-MeOH (8:2). c) $NaOH/H_2O$-MeOH (8:2).

Table 6. Alkylation of Nucleosides with Dienone **3**

nucleoside	products (yield)	
 **22**	 **26** (0.3%)	 **27** (0.4%)
 **23**	 **28** (0.3%)	 **29** (0.5%)
 **24**	 **30** (2.7%)	 **31** (0.9%)
 **25**	 **32** (2.3%)	



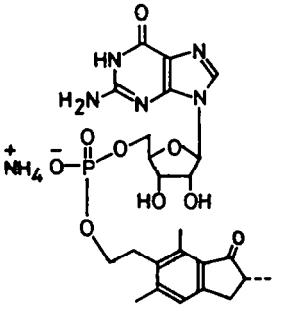


33

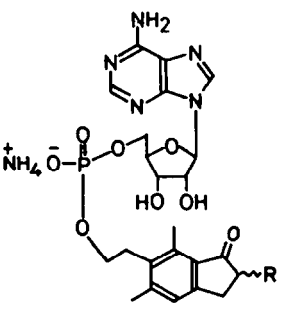
alkylated at the 5'-OH position of the ribose moiety. The site of alkylation of the ribose moiety in **27** was determined on the basis of the acetylation shift observed in the ^1H NMR spectra of **27** and the derived diacetate **33**. Guanosine (**23**) afforded two alkylation products, **28** (0.3%) and **29** (0.5%). While the product **28** was proved to be alkylated at the O^6 position of guanine, the product **29** was found to be a zwitter ionic compound formed by alkylation at the N-7 position of guanine. In the case of uridine (**24**) there were obtained the product **30** (2.7%) alkylated at the N-3 position of uracil and the product **31** (0.9%) with alkylation at the O^4 position of uracil. Cytidine (**25**) provided a product **32** (2.3%) alkylated at the N-3 position of cytosine.

Reaction of Dienone 3 with Nucleotides

Two nucleotides, 5'-guanylic acid (5'-GMP) and 5'-adenylic acid (5'-AMP) were reacted with dienone **3** under the same conditions as those employed in the case of nucleosides. Both nucleotides afforded the products, **34** (30%) and **35** (28%) alkylated at the phosphate group, respectively. The product **35** was shown to consist of two diastereomers (**35a** and **35b**, the ratio of 2:1) regarding the secondary methyl group in the indanone moiety. The site of alkylation in both products (**34** and **35**) was determined to be the phosphate group by the enzymatic hydrolysis with snake venom diesterase, which provided pterosin B (**4**) and the starting nucleotide (5'-GMP and 5'-AMP, respectively).



34



35a R = α-Me
35b R = β-Me

From these findings the reactivities of dienone **3** as an alkylating agent toward nucleosides and nucleotides are disclosed, and these results could be useful for the structural analysis of DNA chemically modified by dienone **3**.

Experimental

Melting points are uncorrected. UV spectra were taken on a JASCO UVIDEC-510 spectrophotometer. IR spectra were obtained with either a JASCO Model IR-S or a JASCO Model IR-810 spectrophotometer. ^1H NMR spectra were recorded on JEOL GX-270 (270 MHz), JEOL GX-400 (400 MHz), and JEOL GX-500 (500 MHz) instruments: chemical shifts (δ) are reported in ppm downfield from internal TMS in organic solutions and from internal DSS in D_2O solutions; coupling constants are reported in Hz. ^{13}C NMR spectra were recorded on a JEOL GX-270 (67.8 MHz) spectrometer: chemical shifts (δ) are reported in ppm downfield from internal TMS. The low resolution mass spectra (EIMS, DEIMS, DCIMS, and FABMS) and the high resolution mass spectra (HREIMS, HRDEIMS, HRDCIMS, and HRFABMS) were measured on a JEOL JMS-DX303 instrument. Optical rotations were measured on a JASCO DIP-181 polarimeter.

Fuji-Davison silica gel BW-820 MH was used for column chromatography. Merck precoated silica gel 60 F_{254} plates, 0.25 mm thickness, were used for thin layer chromatography (TLC). HPLC was performed with either a TRI ROTAR-II or a TRI ROTAR-VI apparatus with refractive index (RI) and UV detectors. Unless otherwise indicated, organic solutions were washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure by a rotary evaporator.

Ptaquiloside (**1**). The plants of P. aquilinum var. latiusculum were collected in July in the Nayoro area of Hokkaido, Japan. The dried, finely powdered plant materials (3.0 kg) were heated in H_2O (30 l) for 10 min, rapidly cooled to room temperature with ice-bath, and filtered with suction. The plant materials were further extracted by repeating the procedure described above twice. The combined

aqueous extracts **Fr-I** (ca. 80 l) were evaporated under reduced pressure below 40 °C. The concentrated aqueous extracts (8 l), after being left overnight at 4 °C, were filtered through a pad of Celite, which was washed with H_2O (30 l). The filtrate and the washings were combined and concentrated under reduced pressure below 40 °C. To the resulting aqueous solution (8 l) was added the resin Amberlite XAD-2 (wet volume, 9 l). The mixture was stirred at room temperature for 1 h, filtered, and washed with H_2O (20 l). The filtrate and the washings were combined and evaporated under reduced pressure below 40 °C. The concentrated aqueous solution (8 l) was again treated with fresh Amberlite XAD-2 (wet volume, 9 l) and the mixture, after being stirred at room temperature for 1 h, was filtered. To the combined Amberlite XAD-2 (18 l) was added 3:7 MeOH-H_2O (20 l). The mixture was stirred at room temperature for 3 h, filtered, and washed with H_2O (20 l). Then, MeOH (32 l) was added to the resin Amberlite XAD-2. The resulting mixture was stirred at room temperature for 3 h, left overnight at room temperature, filtered, and washed with MeOH (32 l). After the filtrate and the washings were combined, the MeOH solution was concentrated and freeze-dried to give a solid **Fr-II** (43 g). A solution of the solid **Fr-II** (43 g) in n-BuOH saturated with H_2O (650 ml) was extracted with H_2O saturated with n-BuOH (3 x 650 ml). The combined aqueous extracts (1950 ml) was concentrated under reduced pressure and freeze-dried to afford an amorphous solid **Fr-III** (28 g). A solution of the solid **Fr-III** (28 g) in H_2O saturated with n-BuOH (600 ml) was extracted with n-BuOH saturated with H_2O (20 x 250 ml). Concentration of the combined n-BuOH extracts and subsequent freeze-drying of the residue provided a solid **Fr-IV** (19 g). To the solution of the solid **Fr-IV** (19 g) in 2:8 MeOH-H_2O (1 l) was added the resin Toyopearl HW-40(c) (wet volume, 500 ml). The mixture was stirred at room temperature for 2 h, filtered, and washed with H_2O (2 x 250 ml and 500 ml). The filtrate and the washings were combined, and the mixture was concentrated and freeze-dried to give a solid **Fr-V** (12 g). Silica gel (4 g) impregnated with the solid **Fr-V** (2 g) was placed on the top of the silica gel (40 g) column. The column was eluted successively with $CHCl_3$-MeOH [5:1 (480 ml), 3:1 (480 ml), 1:1 (120 ml)] and MeOH (120 ml). The fractions eluted with 5:1 $CHCl_3$-MeOH were concentrated and freeze-dried to give an orange powder (227 mg). Chromatography of the powder (227 mg) using a column of Develosil ODS 30/60 (300 x 22 mm I.D.) with 4:6 MeOH-H_2O under low pressure (flow rate 9.9 ml/min) afforded the fraction of **1** (retention time 64 min), which was concentrated and freeze-dried to provide pure **1** (68 mg, 0.014%) as a colorless amorphous powder: the data of IR and $[\alpha]_D$ are described in the text; ^1H NMR (Table 2); ^{13}C NMR (Table 3) [HRFABMS. Found: 421.1849 $(M + Na)^+$. $C_{20}H_{30}O_8Na$ requires: 421.1838].

Ptaquiloside Tetraacetate (2). A mixture of **1** (25 mg), Ac_2O (0.5 ml), and pyridine (0.5 ml) was stirred at room temperature for 2 h. Concentration of the mixture afforded an amorphous residue, purification of which by chromatography on silica gel (1:2 EtOAc-hexane) afforded **2** (35 mg) as a crystalline solid. Recrystallization from MeOH provided pure **2** (27 mg, 76%) as colorless crystals: mp 173-174 °C (dec.); $[\alpha]_D^{16}$ -169° (c 0.729, $CHCl_3$); IR (KBr) 3450, 1761, 1724 (shoulder), 1640 cm^{-1}; ^1H NMR (Table 2); ^{13}C NMR (Table 3); FABMS m/z 589 $(M + Na)^+$, 567 $(M + H)^+$, 549 $(M + H - H_2O)^+$ [HRFABMS. Found: 567.2464 $(M + H)^+$. $C_{28}H_{39}O_{12}$ requires: 567.2441].

Dienone 3. A mixture of **1** (100 mg) in 0.02 M Na_2CO_3 solution (5 ml) was stirred at 25 °C for 20 min and extracted with 2:1 hexane-ether (4 x 5 ml). The combined organic extracts were passed through a column of anhydrous Na_2CO_3 and concentrated below 25 °C to afford **3** (52 mg, 95%) as a colorless oil: UV (MeCN) 321 (ε 9800), 213 nm (8040); IR (CCl_4) 3480, 1667, 1570 cm^{-1}; ^1H NMR (Table 2); EIMS m/z 218 (M^+), 203, 187, 175 [HREIMS. Found: 218.1309 (M^+). $C_{14}H_{18}O_2$ requires: 218.1307].

Detection of D-(+)-Glucose from Ptaquiloside (1). A mixture of **1** (20 mg) in 0.01 M Na_2CO_3 solution (2 ml) was stirred at 20 °C for 20 min and extracted with EtOAc (2 x 2 ml). The aqueous layer was neutralized with 1 N HCl and concentrated under reduced pressure to give a solid. A mixture of the solid and conc. H_2SO_4 (15 μl) in MeOH (4 ml) was refluxed for 12 h, cooled to room temperature, neutralized with saturated aqueous $NaHCO_3$ solution, and evaporated under reduced pressure to leave a solid. A mixture of the solid in Ac_2O (1 ml) and pyridine (1 ml) was stirred at room temperature for 12 h and concentrated. The resulting residue was extracted with $CHCl_3$ (10 ml). The $CHCl_3$ extract was concentrated to give an oily residue, which was separated by preparative TLC on silica gel (5:1 CCl_4-acetone) to give methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (7.6 mg, 42%) and its β-anomer (4.6 mg, 25%). Methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside: mp 65-66 °C (ether-hexane); $[\alpha]_D^{21}$ +131° (c 0.77, $CHCl_3$). The authentic sample prepared from D-(+)-glucose (i. MeOH-H_2SO_4, reflux; ii. Ac_2O-pyridine, room temperature) gave mp 65.0-66.5 °C; $[\alpha]_D^{22}$ +137° (c 0.47, $CHCl_3$).

Pterosin B (4) from Dienone 3. Chromatography of **3** (9 mg) on silica gel (1 g) (4:1 benzene-EtOAc) afforded **4** (6.4 mg, 71%): mp 109.0-109.5 °C (hexane-$CHCl_3$); $[\alpha]_D^{25}$ -44° (c 1.00, MeOH) [lit.[6b] mp 109-110 °C; $[\alpha]_D$ -31.9° (c 0.31, MeOH)].

General Procedure for Reactions of Dienone 3 with Amino Acids. The 0.133 M aqueous solution of an amino acid was prepared, the pH of which was adjusted to 7.5 by adding a trace amount of 1 N NaOH. The 0.20 M acetone solution of **3** (1/3 volume of the aqueous amino acid solution) was added to the aqueous amino acid solution [the 0.20 M THF solution of **3** was used in the case of cysteine (**9**)]: the concentrations of the amino acid and **3** in the resulting mixture were 0.10 M and 0.05 M, respectively. The mixture was stirred at 37 °C for 2 h under an argon atmosphere. The individual products were isolated by the workup of the reaction mixture described in each case. The yield of the product was based on dienone **3**.

Reaction of 3 with Alanine (8). The reaction mixture obtained from **3** (218 mg, 1.00 mmol) and alanine (**8**) (178 mg, 2.00 mmol) was concentrated under reduced pressure and extracted with $CHCl_3$ (4 x 15 ml). The combined organic extracts were dried and concentrated to give an oil (208 mg). Separation by chromatography on silica gel (1:1 → 1:2 benzene-acetone) gave pterosin B (**4**) (168 mg, 77%) and the ester **12** (10.1 mg, 3.5%), respectively. **12**: oil; $[\alpha]_D^{20}$ -19.7° (c 0.833, $CHCl_3$); IR

($CHCl_3$) 3380, 3320, 1732, 1696, 1600, 1184, 972, 882 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.28 (3H, d, J = 7.3), 1.33 (3H, d, J = 7.3), 1.62 (2H, br s), 2.45 (3H, s), 2.60 (2H, m), 2.70 (3H, s), 3.07 (2H, t, J = 7.9), 3.25 (1H, dd, J = 16.5, 7.6), 3.55 (1H, q, J = 7.3), 4.19 (2H, t, J = 7.9), 7.11 (1H, s); EIMS m/z 289 (M^+), 231, 202, 201, 187 [HREIMS. Found: 289.1693 (M^+). $C_{17}H_{23}NO_3$ requires: 289.1678].

Reaction of **3** with Cysteine (**9**). The reaction mixture obtained from **3** (55 mg, 0.25 mmol) and cysteine (**9**) (61 mg, 0.50 mmol) was diluted with H_2O (5 ml) and extracted with n-BuOH (3 x 10 ml). The combined organic extracts were concentrated to give an oil (72 mg), which was chromatographed on silica gel (1:1 benzene-EtOAc, EtOAc, 95:4.5:0.5 → 90:9:1 → 80:18:2 → 70:27:3 → 60:36:4 EtOAc-MeOH-H_2O). The fraction eluted with 1:1 benzene-EtOAc afforded pterosin B (**4**) (39 mg, 72%). The fraction eluted with 90:9:1 EtOAc-MeOH-H_2O gave the crude ester **14** (2.2 mg), which was further purified by preparative TLC on silica gel (5:4:1 hexane-EtOAc-MeOH) to provide **14** (1.1 mg, 1.4%). From the fraction eluted with 80:18:2 → 60:36:4 EtOAc-MeOH-H_2O, a mixture of cysteine (**9**) and the S-alkylated product **13** was obtained, separation of which by HPLC [column, Develosil ODS 10/20 (250 x 20 mm I.D.); solvent, 60:40 MeOH-0.02 M NH_4OAc; flow rate, 5.0 ml/min; retention time, 37.3 min] yielded **13** (16.7 mg, 21%). **13**: mp 186-189 °C (MeOH-H_2O); $[\alpha]_D^{22}$ -12.9° (c 0.786, 1:1 MeOH-0.2 N NH_4OH); IR (KBr) 3500-2300 (broad), 1697, 1629, 1600, 1218, 1130, 967, 883 cm^{-1}; ^1H NMR (500 MHz, 4:1 C_5D_5N-D_2O) δ 1.29 (3H, d, J = 7.3), 2.42 (3H, s), 2.49 (1H, dd, J = 17.2, 3.9), 2.63 (1H, m), 2.69 (3H, s), 2.88 (2H, m), 3.00 (2H, m), 3.19 (1H, dd, J = 17.2, 8.0), 3.57 (1H, br s), 3.83 (1H, br d, J = 12.8), 4.44 (1H, br s), 7.00 (1H, s); FABMS m/z 322 (M + H)$^+$ (Found: C, 60.69; H, 7.10; N, 4.14. $C_{17}H_{23}NO_3S \cdot H_2O$ requires: C, 60.15; H, 7.42; N, 4.13%). **14**: oil; $[\alpha]_D^{20}$ -24° (c 0.18, $CHCl_3$); IR ($CHCl_3$) 3690, 3380(broad), 1735, 1695, 1600, 1220 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.27 (3H, d, J = 7.3), 2.44 (3H, s), 2.60 (2H, m), 2.70 (3H, s), 2.89 (1H, dd, J = 13.7, 7.9), 3.08 (2H, t, J = 7.9), 3.11 (1H, dd, J = 13.7, 4.6), 3.24 (1H, dd, J = 16.8, 7.9), 3.81 (1H, dd, J = 7.6, 4.6), 4.21 (2H, m), 7.10 (1H, s); EIMS m/z 321 (M^+), 243, 226, 201, 198, 187, 64 [HREIMS. Found: 321.1425 (M^+). $C_{17}H_{23}NO_3S$ requires: 321.1399].

Reaction of **3** with Methionine (**10**). The reaction mixture obtained from **3** (109 mg, 0.50 mmol) and methionine (**10**) (149 mg, 1.00 mmol) was diluted with H_2O (10 ml) and extracted with $CHCl_3$ (4 x 20 ml). The combined organic extracts were dried and concentrated to give an oil (76 mg), which was chromatographed on silica gel (9:1 → 7:1 benzene-EtOAc, EtOAc, 98:2 → 95:5 → 90:10 EtOAc-MeOH). The fraction eluted with 7:1 benzene-EtOAc gave the crude S-alkylated product **16** (3.5 mg), which was purified by preparative TLC on silica gel (2:1 $CHCl_3$-hexane) to afford **16** (1.0 mg, 0.8%) as crystals. The fraction eluted with EtOAc provided pterosin B (**4**) (67 mg, 61%). From the fraction eluted with 95:5 → 90:10 EtOAc-MeOH the ester **17** (5.1 mg, 2.9%) was obtained. The aqueous phase (ca. 18 ml) obtained after extraction of the reaction mixture with $CHCl_3$ was passed through a column of the ion-exchange resin Amberlite CG 50 (H form) (5 g). After being washed with H_2O (50 ml), the ion-exchange resin column was eluted with 0.1 N HCl (100 ml) and 0.2 N HCl (100 ml). Concentration of the resulting aqueous solution under reduced pressure afforded an oily residue. The residue was dissolved in H_2O (20 ml) and the solution was evaporated under reduced pressure; this process was repeated three times to leave a viscous oil, freeze-drying of which yielded the sulfonium salt **15** (49 mg, 23%). **15**: pale yellow powder; $[\alpha]_D^{22}$ +15.6° (c 0.53, MeOH); IR (KBr) 3500-2300 (broad), 1745, 1692, 1601, 1219, 1197, 885 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.22 (3H, d, J = 7.3), 2.41 (2H, m), 2.45 (3H, s), 2.62 (3H, s), 2.66 (1H, dd, J = 17.5, 3.5), 2.75 (1H, ddq, J = 8.5, 3.5, 7.3), 2.99 (3H, s), 3.29 (2H, t, J = 8.2), 3.31 (1H, dd, J = 17.5, 8.2), 3.40-3.60 (3H, m), 3.65 (1H, dt, J = 12.5, 8.2), 3.96 (1H, t, J = 6.3), 7.31 (1H, s); FABMS m/z 350 (M + H)$^+$ [HRFABMS. Found: 350.1781 (M + H)$^+$. $C_{19}H_{28}NO_3S$ requires: 350.1790]. **16**: mp 50-54 °C (pentane); IR ($CHCl_3$) 1692, 1601, 881 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.27 (3H, d, J = 7.3), 2.21 (3H, s), 2.41 (3H, s), 2.56 (2H, m), 2.60 (2H, m), 2.67 (3H, s), 2.98 (2H, m), 3.24 (1H, dd, J = 16.8, 7.7), 7.09 (1H, s); EIMS m/z 248 (M^+), 233, 200, 187 [HREIMS. Found: 248.1247 (M^+). $C_{15}H_{20}OS$ requires: 248.1235]. **17**: oil; $[\alpha]_D^{20}$ -24.9° (c 0.43, $CHCl_3$); IR ($CHCl_3$) 3360 (broad), 1732, 1692, 1601, 1176, 972, 884 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.28 (3H, d, J = 7.3), 1.80 (1H, m), 2.02 (1H, m), 2.10 (3H, s), 2.45 (3H, s), 2.59 (2H, t, J = 7.4), 2.60 (2H, m), 2.70 (3H, s), 3.07 (2H, t, J = 7.7), 3.25 (1H, dd, J = 16.7, 7.4), 3.59 (1H, dd, J = 8.4, 5.3), 4.20 (2H, t, J = 7.7), 7.11 (1H, s); EIMS m/z 349 (M^+), 202, 201, 187 [HREIMS. Found: 349.1707 (M^+). $C_{19}H_{27}NO_3S$ requires: 349.1712].

Reaction of **3** with Glutathione (**11**). The reaction mixture obtained from **3** (55 mg, 0.25 mmol) and glutathione (**11**) (154 mg, 0.50 mmol) was diluted with H_2O (5 ml) and extracted with $CHCl_3$ (3 x 10 ml). The $CHCl_3$ extracts were dried and concentrated to afford pterosin B (**4**) (38 mg, 70%). During the extraction a solid precipitated, which was filtered to give a colorless powder (27 mg). For the complete removal of $CHCl_3$ and acetone, the aqueous phase (ca. 10 ml) was concentrated. To the resulting aqueous solution (ca. 5 ml) was dissolved the colorless powder (27 mg) described above. The aqueous solution was subjected to low pressure liquid chromatographic separation [column, Develosil ODS 30/60 (300 x 22 mm I.D.); solvent, MeOH-0.02 M NH_4OAc [50:50 (10 min), 50:50 → 70:30 (linear gradient, 25 min), 70:30 (20 min)]; flow rate, 9.9 ml/min] to afford the ammonium salt of **18** (10.6 mg, 8.1%, retention time 35.0 min), the ester **20** (2.8 mg, 2.2%, retention time 42.1 min), and the impure ester **19** (5.0 mg, retention time 51.0 min), respectively. Further purification of impure **19** by HPLC [column, Develosil ODS 10/20 (250 x 20 mm I.D.); solvent, 65:35 MeOH-0.02 M NH_4OAc; flow rate, 6.0 ml/min] gave **19** (3.1 mg, 2.4%, retention time 25.6 min). An aqueous solution of the ammonium salt of **18** was acidified (pH ca. 3) with 0.1 N HCl and the resulting precipitates were filtered and washed with H_2O to afford **18** as crystals. **18**: mp 193-196 °C (MeOH-H_2O); $[\alpha]_D^{22}$ -44.5° (c 0.55, 1:1 MeOH-0.2 N NH_4OH); IR (KBr) 3700-2400 (broad), 1729 (shoulder), 1692, 1668, 1644, 1600 cm^{-1}; ^1H NMR (500 MHz, 4:1 C_5D_5N-D_2O) δ 1.29 (3H, d, J = 7.3), 2.44 (3H, s), 2.49 (1H, dd, J = 17.2, 3.9), 2.64 (1H, m), 2.69 (1H, m), 2.72 (3H, s), 2.80 (3H, m), 2.98 (3H, m), 3.15 (1H, m), 3.20 (1H, dd, J = 17.2, 7.8), 3.34 (1H, dd, J = 13.8, 9.2), 3.61 (1H, dd, J = 13.8, 5.0), 4.30 (1H, d, J = 17.4), 4.37 (1H, d, J = 17.4), 5.22 (1H, dd, J = 9.2, 5.0), 7.03 (1H, s); FABMS m/z 508 (M + H)$^+$ (Found: C, 54.48; H, 6.46; N, 7.95. $C_{24}H_{33}N_3O_7S \cdot H_2O$ requires: C, 54.84; H, 6.71; N, 7.99%). **19**: amorphous powder; $[\alpha]_D^{22}$ -17° (c 0.26, 1:1 MeOH-0.2 N NH_4OH); IR (KBr) 3600-2400 (broad), 1741, 1694, 1639, 1601, 1200 cm^{-1}; ^1H NMR (500 MHz, 4:1 C_5D_5N-D_2O) δ 1.29 (3H, d, J = 7.3), 2.39 (3H, s), 2.50 (1H, dd, J =

17.1, 3.7), 2.65 (2H, m), 2.69 (3H, s), 2.80 (1H, m), 2.97 (1H, dt, J = 15.2, 7.5), 3.02 (2H, t, J = 7.7), 3.12 (1H, dt, J = 15.2, 7.5), 3.21 (1H, dd, J = 17.1, 7.9), 3.28 (2H, d, J = 6.1), 4.22 (2H, t, J = 7.7), 4.27 (1H, d, J = 17.7), 4.33 (1H, m), 4.34 (1H, d, J = 17.7), 5.14 (1H, t, J = 6.1), 7.04 (1H, s); FABMS m/z 508 $(M + H)^+$. **20**: amorphous powder; $[\alpha]_D^{22}$ -43° (c 0.23, 1:1 MeOH-0.2 N NH_4OH); IR (KBr) 3600-2400 (broad), 1741, 1695, 1653, 1600, 1222 cm^{-1}; ^1H NMR (500 MHz, 4:1 C_5D_5N-D_2O) δ 1.30 (3H, d, J = 7.3), 2.45 (1H, m), 2.48 (3H, s), 2.52 (1H, dd, J = 17.4, 3.7), 2.55 (1H, m), 2.66 (1H, ddq, J = 8.5, 3.7, 7.3), 2.74 (3H, s), 2.89 (1H, dt, J = 15.2, 7.5), 2.99 (1H, dt, 15.2, 7.5), 3.13 (2H, t, J = 8.0), 3.23 (1H, dd, J = 17.4, 8.5), 3.25 (1H, dd, J = 14.0, 7.4), 3.31 (1H, dd, J = 14.0, 5.0), 4.17 (1H, dd, J = 7.5, 6.0), 4.22 (1H, d, J = 17.1), 4.34 (1H, d, J = 17.1), 4.34 (2H, t, J = 8.0), 5.12 (1H, dd, J = 7.4, 5.0), 7.07 (1H, s); FABMS m/z 508 $(M + H)^+$.

Reaction of Ptaquiloside (**1**) with Methionine (**10**) at pH 9.2: Isolation of Homoserine (**21**). A mixture of **1** (40 mg, 0.10 mmol) and methionine (**10**) (75 mg, 0.50 mmol) in 0.25 N NaOH solution (1 ml) was stirred at 37 °C for 12 h, neutralized with 1 N HCl (0.25 ml), and extracted with $CHCl_3$ (3 x 3 ml). The combined $CHCl_3$ extracts were dried and concentrated to give an oil (11 mg), which was chromatographed on silica gel (4:1 $CHCl_3$-EtOAc, EtOAc, 95:5 EtOAc-MeOH). The fractions eluted with 4:1 $CHCl_3$-EtOAc afforded **16** (2.5 mg, 12%) and pterosin B (**4**) (5.6 mg, 26%). From the fraction eluted with 95:5 EtOAc-MeOH, the ester **17** (1.4 mg, 4%) was obtained. The aqueous phase was passed through a column of the ion-exchange resin Amberlite CG 50 (H form) (2.4 g), and then the column was eluted with H_2O and HCl (0.05 N → 0.1 N → 0.2 N), successively. From the fraction eluted with H_2O, a mixture (89 mg) of methionine (**10**) and homoserine (**21**) was obtained, which was separated by chromatography on silica gel (5:4:1 $CHCl_3$-MeOH-H_2O) to give homoserine (**21**) (1.2 mg, 10%) as a colorless powder. The ^1H NMR spectral data and chromatographic properties of **21** were identical with those of authentic homoserine. The fractions eluted with dil. hydrochloric acid afforded **15** (17.5 mg, 41%) as a pale yellow powder.

General Procedure for Reactions of Dienone **3** with Nucleosides and Nucleotides. The 0.133 M aqueous solution of a nucleoside or a nucleotide was prepared, the pH of which was adjusted to 7.5 by adding a trace amount of 1 N NaOH or 1 N HCl. To the aqueous solution was added the 0.20 M acetone solution of **3** (1/3 volume of the aqueous solution of the nucleoside or the nucleotide): the concentrations of **3** and the nucleoside (or the nucleotide) in the mixture were 0.10 M and 0.05 M, respectively. In the case of guanosine (**23**), the concentrations of **3** and **23** were 0.0125 M, respectively, owing to the low solubility of **23**. The mixture was stirred at 37 °C for 2 h. The individual products were isolated by the workup of the reaction mixture described in each case.

Reaction of **3** with Adenosine (**22**). The reaction mixture obtained from **3** (55 mg, 0.25 mmol) and adenosine (**22**) (134 mg, 0.50 mmol) was diluted with H_2O (5 ml) and extracted with $CHCl_3$ (4 x 10 ml). The combined $CHCl_3$ extracts were dried and concentrated to give an oil (53 mg), which was chromatographed on silica gel (99:1 → 80:20 $CHCl_3$-MeOH). From the fractions eluted with 96:4 → 92:8 $CHCl_3$-MeOH, pterosin B (**4**) (49 mg, 90%) was obtained. The fractions eluted with 90:10 → 85:15 $CHCl_3$-MeOH gave a mixture of two products, separation of which by preparative TLC on silica gel (2:2:1 hexane-EtOAc-MeOH) provided **26** (0.4 mg, 0.3%) and **27** (0.5 mg, 0.4%). **26**: amorphous powder; UV (MeOH) 215 (ε 42100), 262 (29200), 302 nm (2600, shoulder); difference UV (Table 5); IR (KBr) 3400, 1698, 1618, 1600, 1225, 1080 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.23 (3H, d, J = 7.3), 2.51 (3H, s), 2.60 (2H, m), 2.70 (3H, s), 3.12 (2H, m), 3.74 (1H, dd, J = 12.5, 2.4), 3.89 (1H, dd, J = 12.5, 2.4), 4.18 (1H, dt, J = 2.4, 2.4), 4.32 (1H, dd, J = 5.2, 2.4), 4.75 (1H, dd, J = 6.4, 5.2), 5.94 (1H, d, J = 6.4), 7.15 (1H, s), 8.23 (2H, s); DCIMS m/z 468 $(M + H)^+$, 336, 280, 133 [HRDCIMS. Found: 468.2236 $(M + H)^+$. $C_{24}H_{30}N_5O_5$ requires: 468.2247]. **27**: amorphous powder; UV (MeOH) 213 (ε 48200), 258 (29400), 300 nm (3400); difference UV (Table 5); IR (KBr) 3420, 1692, 1632, 1600, 1110, 1072 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.22 (3H, d, J = 7.3), 2.42 (3H, s), 2.61 (2H, m), 2.63 (3H, s), 3.06 (2H, m), 3.62 (2H, t, J = 7.5), 3.72 (1H, dd, J = 10.9, 3.8), 3.79 (1H, dd, J = 10.9, 3.2), 4.18 (1H, ddd, J = 4.9, 3.8, 3.2), 4.34 (1H, t, J = 4.9), 4.55 (1H, t, J = 4.9), 6.02 (1H, d, J = 4.9), 7.13 (1H, s), 8.20 (1H, s), 8.21 (1H, s); DCIMS m/z 468 $(M + H)^+$, 219, 201, 164, 133, 117, 87 [HRDCIMS. Found: 468.2225 $(M + H)^+$. $C_{24}H_{30}N_5O_5$ requires: 468.2247].
A mixture of **27** (1.0 mg), Ac_2O (0.3 ml), and pyridine (0.6 ml) was stirred at room temperature for 2 h. The residue obtained by concentration of the mixture was chromatographed on silica gel (9:1 EtOAc-MeOH) to give the diacetate **33** (1.1 mg) as an oil: IR ($CHCl_3$) 3530, 3415, 1750, 1700, 1635, 1601, 1240, 1100, 910 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.29 (3H, d, J = 7.3), 2.05 (3H, s), 2.15 (3H, s), 2.43 (3H, s), 2.62 (2H, m), 2.69 (3H, s), 3.09 (2H, m), 3.25 (1H, dd, J = 17.0, 7.9), 3.60 (2H, t, J = 7.3), 3.77 (2H, m), 4.36 (1H, dt, J = 3.0, 3.0), 5.62 (1H, dd, J = 5.4, 3.0), 6.40 (1H, dd, J = 6.4, 5.4), 6.31 (1H, d, J = 6.4), 7.10 (1H, s), 8.04 (1H, s), 8.37 (1H, s); DCIMS m/z 552 $(M + H)^+$, 510, 417 [HRDCIMS. Found: 552.2455 $(M + H)^+$. $C_{28}H_{34}N_5O_7$ requires: 552.2458].

Reaction of **3** with Guanosine (**23**). The reaction mixture obtained from **3** (109 mg, 0.50 mmol) and guanosine (**23**) (142 mg, 0.50 mmol) was extracted with 2:1 ether-hexane (3 x 40 ml). On evaporation of the combined organic extracts pterosin B (**4**) (103 mg, 94%) was obtained. The aqueous phase was extracted with n-BuOH (5 x 30 ml). The combined n-BuOH extracts were concentrated to give an oily residue (27 mg), column chromatography of which on silica gel with $CHCl_3$-MeOH-H_2O (100:0:0 → 0:90:10, linear gradient, 64 min, flow rate 2 ml/min) provided **28** (0.7 mg, 0.3%) and the mixture of **29** and guanosine (**23**). The mixture was further separated by HPLC [column, Develosil ODS 10/20 (250 x 20 mm I.D.); solvent, 30:70 MeCN-0.02 M NH_4OAc; flow rate, 6.0 ml/min] to give **29** (1.2 mg, 0.5%, retention time 17.0 min). **28**: amorphous powder; UV (MeOH) 213 (ε 67100), 252 (29100), 281 nm (15600); difference UV (Table 5); IR (KBr) 3400, 1690, 1611, 1589, 1512, 787 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.23 (3H, d, J = 7.3), 2.51 (3H, s), 2.62 (2H, m), 2.71 (3H, s), 3.73 (1H, dd, J = 12.3, 2.7), 3.88 (1H, dd, J = 12.3, 2.7), 4.13 (1H, dt, J = 2.7, 2.7), 4.31 (1H, dd, J = 5.2, 2.7), 4.63 (2H, t, J = 7.5), 4.70 (1H, dd, J = 6.4, 5.2), 5.84 (1H, d, J = 6.4), 7.16 (1H, s), 8.02 (1H, s); DEIMS m/z 483 (M^+), 453, 394, 351, 321, 218, 203, 200, 187 [HRDEIMS. Found: 483.2082 (M^+). $C_{24}H_{29}N_5O_6$ requires: 483.2118]. **29**: amorphous powder; UV (MeOH) 216 (ε 59400), 258 (33000), 284 nm (13200, shoulder); difference UV (Table 5); IR (KBr) 3350, 1700, 1636, 1599, 1525, 1200, 770 cm^{-1}; ^1H NMR

(270 MHz, CD_3OD) δ 1.25 (3H, d, J = 7.2), 2.46 (3H, s), 2.62 (3H, s), 2.62 (2H, m), 3.40 (2H, t, J = 7.7), 3.78 (1H, dd, J = 12.3, 2.3), 3.92 (1H, dd, J = 12.3, 2.3), 4.13 (1H, dt, J = 6.0, 2.3), 4.24 (1H, dd, J = 6.0, 4.6), 4.40 (1H, dd, J = 4.6, 3.0), 4.54 (2H, m), 5.97 (1H, d, J = 3.0), 7.23 (1H, s), 9.33 (1H, s); FABMS m/z 484 $(M + H)^+$ [HRFABMS. Found: 484.2214 $(M + H)^+$. $C_{24}H_{30}N_5O_6$ requires: 484.2196].

Reaction of **3** with Uridine (**24**). The reaction mixture obtained from **3** (55 mg, 0.25 mmol) and uridine (**24**) (122 mg, 0.50 mmol) was diluted with H_2O (5 ml) and extracted with EtOAc (3 x 10 ml). The combined organic extracts were dried and concentrated to give an oily residue (57 mg), which was chromatographed on silica gel (98:2 → 80:20 $CHCl_3$-MeOH). The fraction eluted with 96:4 $CHCl_3$-MeOH gave pterosin B (**4**) (49 mg, 90%). From the fractions eluted with 92:8 → 85:15 $CHCl_3$-MeOH, there was obtained a mixture of the products, which was separated by preparative TLC on silica gel (5:1 $CHCl_3$-MeOH) to afford **30** (3.0 mg, 2.7%) and crude **31** (1.3 mg). Purification of crude **31** by preparative TLC on silica gel (8:1 EtOAc-MeOH) yielded **31** (1.0 mg, 0.9%). **30**: amorphous powder; UV (MeOH) 217 (ε 40500), 259 (24800), 300 nm (2800); difference UV (Table 5); IR (KBr) 3520, 3420, 1700, 1682, 1652, 1613, 1600, 1102, 820, 768 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.23 (3H, d, J = 7.1), 2.52 (3H, s), 2.62 (2H, m), 2.72 (3H, s), 3.02 (2H, m), 3.25 (1H, m), 3.74 (1H, dd, J = 12.5, 2.8), 3.87 (1H, dd, J = 12.5, 2.5), 3.97 (2H, m), 4.02 (1H, m), 4.15 (2H, m), 5.78 (1H, d, J = 7.9), 5.92 (1H, d, J = 3.4), 7.17 (1H, s), 8.06 (1H, d, J = 7.9); DCIMS m/z 445 $(M + H)^+$, 313, 200, 61 [HRDCIMS. Found: 445.1994 $(M + H)^+$. $C_{23}H_{29}N_2O_7$ requires: 445.1975]. **31**: amorphous powder; UV (MeOH) 216 (ε 42700), 259 (19600), 278 nm (9800, shoulder); difference UV (Table 5); IR (KBr) 3380, 1705, 1650, 1640 (shoulder), 1602, 1540, 1223, 1072, 1060, 794 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.23 (3H, d, J = 7.2), 2.48 (3H, s), 2.61 (2H, m), 2.68 (3H, s), 3.21 (2H, t, J = 7.4), 3.75 (1H, dd, J = 12.5, 2.8), 3.92 (1H, dd, J = 12.5, 2.5), 4.04 (1H, ddd, J = 5.9, 2.8, 2.5), 4.11 (2H, m), 4.47 (2H, t, J = 7.4), 5.86 (1H, d, J = 2.4), 6.02 (1H, d, J = 7.5), 7.17 (1H, s), 8.40 (1H, d, J = 7.5); DCIMS m/z 445 $(M + H)^+$, 313, 247, 220, 218, 203, 187, 133, 78 [HRDCIMS. Found: 445.1989 $(M + H)^+$. $C_{23}H_{29}N_2O_7$ requires: 445.1974].

Reaction of **3** with Cytidine (**25**). The reaction mixture obtained from **3** (55 mg, 0.25 mmol) and cytidine (**25**) (122 mg, 0.50 mmol) was diluted with H_2O (5 ml) and extracted with n-BuOH (3 x 10 ml). The combined organic extracts were concentrated to give an oil (88 mg), which was chromatographed on silica gel (100:0 → 70:30 $CHCl_3$-MeOH). The fraction eluted with 96:4 $CHCl_3$-MeOH afforded pterosin B (**4**) (50 mg, 92%). The fractions eluted with 85:15 → 70:30 $CHCl_3$-MeOH yielded a mixture of **32** and cytidine (**25**), further separation of which with preparative TLC on silica gel (2:1 $CHCl_3$-MeOH) provided **32** (2.6 mg, 2.3%). **32**: amorphous powder; UV (MeOH) 218 (ε 36700), 260 (19700), 281 nm (8400, shoulder); difference UV (Table 5); IR (KBr) 3400, 1690 (shoulder), 1664, 1598, 1080, 760 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.23 (3H, d, J = 7.1), 2.51 (3H, s), 2.61 (2H, m), 2.70 (3H, s), 3.08 (2H, m), 3.24 (1H, m), 3.71 (1H, dd, J = 12.3, 3.3), 3.82 (1H, dd, J = 12.3, 2.8), 3.96 (1H, ddd, J = 4.5, 3.3, 2.8), 4.00 (1H, t, J = 4.5), 4.08 (1H, t, J = 4.5), 4.10 (2H, m), 5.77 (1H, d, J = 4.5), 5.84 (1H, d, J = 7.9), 7.13 (1H, s), 7.50 (1H, d, J = 7.9); DCIMS m/z 444 $(M + H)^+$, 312, 244, 203, 175, 133, 117 [HRDCIMS. Found: 444.2137 $(M + H)^+$. $C_{23}H_{30}N_3O_6$ requires: 444.2135].

Reaction of **3** with 5'-Guanylic Acid (5'-GMP). The reaction mixture obtained from **3** (55 mg, 0.25 mmol) and 5'-GMP disodium salt containing 17% H_2O by weight (245 mg, 0.50 mmol) was diluted with H_2O (5 ml) and extracted with $CHCl_3$ (3 x 10 ml). Concentration of the combined organic extracts afforded pterosin B (**4**) (31 mg, 57%). The aqueous phase was subjected to low pressure liquid chromatographic separation [column, Develosil ODS 30/60 (300 x 22 mm I.D.); solvent, 20:80 MeCN-0.02 M NH_4OAc; flow rate, 6.0 ml/min] to give **34** (43 mg, 30%, retention time 25.0 min). **34**: amorphous powder; UV (H_2O) 257 (ε 23800), 302 nm (3000, shoulder); IR (KBr) 1685, 1630, 1601, 1533 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 1.18 (3H, d, J = 7.6), 2.20 (3H, s), 2.34 (3H, s), 2.50 (1H, dd, J = 17.1, 3.0), 2.64 (1H, ddq, J = 7.3, 3.0, 7.6), 2.76 (1H, m), 2.85 (1H, m), 3.19 (1H, dd, J = 17.1, 7.3), 3.64 (1H, m), 3.75 (1H, m), 3.88 (2H, m), 4.19 (1H, br s), 4.43 (1H, dd, J = 5.2, 4.0), 5.74 (1H, d, J = 5.8), 6.98 (1H, s), 7.88 (1H, s); FABMS m/z 564 $(M + H)^+$ [HRFABMS. Found: 564.1844 $(M + H)^+$. $C_{24}H_{31}N_5O_9P$ requires: 564.1859].

Reaction of **3** with 5'-Adenylic Acid (5'-AMP). The reaction mixture obtained from **3** (55 mg, 0.25 mmol) and 5'-AMP containing 25% H_2O by weight (231 mg, 0.50 mmol) was diluted with H_2O (5 ml) and extracted with $CHCl_3$ (3 x 10 ml). Concentration of the combined organic extracts yielded pterosin B (**4**) (38 mg, 70%). Separation of the aqueous phase by low pressure liquid chromatography under the same conditions as employed in the case of 5'-GMP afforded the diastereomeric mixture **35** (39 mg, 28%). Two diastereomers **35a** and **35b** (ratio 2:1) were separated by HPLC [column, Develosil ODS-5 (250 x 10 mm I.D.); solvent, 12:88 THF-0.02 M NH_4OAc; flow rate, 4.0 ml/min]. **35a**: amorphous powder; UV (H_2O) 259 (ε 14200), 300 nm (1800, shoulder); IR (KBr) 1690 (shoulder), 1631, 1603, 1225 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.18 (3H, d, J = 7.5), 2.18 (3H, s), 2.30 (3H, s), 2.50 (1H, dd, J = 17.3, 3.8), 2.63 (1H, ddq, J = 8.0, 3.8, 7.5), 2.73 (1H, m), 2.82 (1H, m), 3.17 (1H, dd, J = 17.3, 8.0), 3.52 (1H, m), 3.68 (1H, m), 3.94 (2H, br s), 4.27 (1H, m), 4.48 (1H, dd, J = 5.5, 4.0), 5.95 (1H, d, J = 3.5), 6.89 (1H, s), 8.05 (1H, s), 8.25 (1H, s); FABMS m/z 548 $(M + H)^+$ [HRFABMS. Found: 548.1879 $(M + H)^+$. $C_{24}H_{31}N_5O_8P$ requires: 548.1910]. **35b**: amorphous powder; UV (H_2O) 260 (ε 11500), 300 nm (1300, shoulder); IR (KBr) 1690 (shoulder), 1631, 1603, 1225 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.16 (3H, d, J = 7.3), 2.23 (3H, s), 2.24 (3H, s), 2.50 (1H, br d, J = 17.5), 2.63 (1H, m), 2.75 (1H, m), 2.82 (1H, m), 3.19 (1H, dd, J = 17.5, 7.5), 3.52 (1H, m), 3.69 (1H, m), 3.94 (2H, m), 4.28 (1H, m), 4.48 (1H, dd, J = 4.8, 3.5), 5.97 (1H, d, J = 5.3), 6.97 (1H, s), 8.04 (1H, s), 8.26 (1H, s); FABMS m/z 548 $(M + H)^+$.

Enzymatic Hydrolysis of the 5'-GMP Adduct **34** and the 5'-AMP Adducts, **35a** and **35b**. Snake venom diesterase (15.5 μg, 15.5 μl 50% aqueous glycerol solution; Boehringer Mannheim Yamanouchi) and **34** (4.5 mg, 7.8 μmol) were incubated in H_2O (520 μl)-0.5 M Tris (150 μl)-0.1 M $MgCl_2$ (78 μl) at pH 8.5 for 17 h at 37 °C. The mixture was separated by HPLC [column, Develosil ODS-5 (250 x 4.6 mm I.D.); solvent, MeCN-0.02 M NH_4OAc (10:90 → 70:30, linear gradient, 10 min); flow rate, 1.0 ml/min] to

give pterosin B (**4**) (retention time 11.5 min) and 5'-GMP (retention time 2.4 min). The yield of **4** was determined to be 83% by quantitative HPLC analysis. By the same procedure as described above, the 5'-AMP adduct **35a** and the diastereomer **35b** afforded pterosin B (**4**) and the epimer of pterosin B (**4**), respectively.

Acknowledgments - We are grateful to Professors S. Matsuura and T. Sugimoto, College of General Education, Nagoya University for helpful discussions on the UV spectra of substituted purines and pyrimidines. We thank Mr. K. Matsushita, JEOL Ltd., Tokyo for his aid in obtaining the ^{1}H and ^{13}C NMR spectra. This work was supported in part by a grant from the Yamada Science Foundation (No. 84-1123) and Grant-in-aid for Scientific Research (No. 62470029) from the Ministry of Education, Science, and Culture.

References and Notes

1. I. A. Evans in "Chemical Carcinogens, Second Edition," ed by C. E. Searle; American Chemical Society; Washington, D. C.; 1984; Vol. 2, Chap. 18, pp 1171-1204.
2. I. Hirono, CRC Crit. Rev. Toxicol., **8**, 235 (1981).
3. I. A. Evans and J. Mason, Nature, **208**, 913 (1965).
4. A. M. Pamukcu and J. M. Price, J. Natl. Cancer Inst., **43**, 275 (1969).
5. I. Hirono, C. Shibuya, K. Fushimi, and M. Haga, J. Natl. Cancer Inst., **45**, 179 (1970).
6. a) K. Yoshihira, M. Fukuoka, M. Kuroyanagi, S. Natori, M. Umeda, T. Morohoshi, M. Enomoto, and M. Saito, Chem. Pharm. Bull., **26**, 2346 (1978) and references cited therein.
   b) M. Fukuoka, M. Kuroyanagi, K. Yoshihira, and S. Natori, Chem. Pharm. Bull., **26**, 2365 (1978) and references cited therein.
7. I. A. Evans in "Chemical Carcinogens," ed by C. E. Searle; American Chemical Society; Washington, D. C.; 1976; Chap. 13, pp 690-700.
8. I. Hirono, K. Yamada, H. Niwa, Y. Shizuri, M. Ojika, S. Hosaka, T. Yamaji, K. Wakamatsu, H. Kigoshi, K. Niiyama, and Y. Uosaki, Cancer Lett., **21**, 239 (1984).
9. H. Niwa, M. Ojika, K. Wakamatsu, K. Yamada, I. Hirono, and K. Matsushita, Tetrahedron Lett., **24**, 4117 (1983).
10. H. Niwa, M. Ojika, K. Wakamatsu, K. Yamada, S. Ohba, Y. Saito, I. Hirono, and K. Matsushita, Tetrahedron Lett., **24**, 5371 (1983).
11. S. Ohba, Y. Saito, I. Hirono, H. Niwa, M. Ojika, K. Wakamatsu, and K. Yamada, Acta Cryst. C**40**, 1877 (1984).
12. I. Hirono, S. Aiso, T. Yamaji, H. Mori, K. Yamada, H. Niwa, M. Ojika, K. Wakamatsu, H. Kigoshi, K. Niiyama, and Y. Uosaki, Gann, **75**, 833 (1984).
13. I. Hirono, Y. Kono, K. Takahashi, K. Yamada, H. Niwa, M. Ojika, H. Kigoshi, K. Niiyama, and Y. Uosaki, Veterinary Record, **115**, 375 (1984).
14. M. Ojika, H. Kigoshi, H. Kuyama, H. Niwa, and K. Yamada, J. Nat. Prod., **48**, 634 (1985).
15. Preliminary communications appeared: ref. 9 and 10.
16. a) H. Hikino, T. Takahashi, S. Arihara, and T. Takemoto, Chem. Pharm. Bull., **18**, 1488 (1970).
    b) K. Yoshihira, M. Fukuoka, M. Kuroyanagi, and S. Natori, Chem. Pharm. Bull., **19**, 1491 (1971).
17. Pterosin B (**4**) obtained from ptaquiloside (**1**) was levorotatory. The absolute configuration at C-2 of (-)-**4** was proved to be R: see M. Kuroyanagi, M. Fukuoka, K. Yoshihira, and S. Natori, Chem. Pharm. Bull., **22**, 723 (1974) and ref. 6b. Therefore, the absolute configuration at C-2 of **1** is identical with that of (-)-**4** previously reported.
18. M. Kuroyanagi, M. Fukuoka, K. Yoshihira, and S. Natori, Chem. Pharm. Bull., **22**, 2762 (1974).
19. H. Seto, T. Sasaki, H. Yonehara, and J. Uzawa, Tetrahedron Lett., 923 (1978).
20. J. C. M. van der Hoeven, W. J. Lagerweij, M. A. Posthumus, A. van Veldhuizen, and H. A. J. Holterman, Carcinogenesis, **4**, 1587 (1983).
21. S. Nozoe in "Natural Products Chemistry," ed by K. Nakanishi, T. Goto, S. Ito, S. Natori, and S. Nozoe; Kodansha, Academic Press; Tokyo, New York, London; 1974; Vol. 1, Chap. 3, pp 90-92.
22. W. A. Ayer and R. H. McCaskill, Can. J. Chem., **59**, 2150 (1981).
23. T. C. McMorris and M. Anchel, J. Am. Chem. Soc., **87**, 1594 (1965).
24. K. Nakanishi, M. Ohashi, M. Tada, and Y. Yamada, Tetrahedron, **21**, 1231 (1965).
25. T. Matsumoto, H. Shirahama, A. Ichihara, Y. Fukuoka, Y. Takahashi, Y. Mori, and M. Watanabe, Tetrahedron, **21**, 2671 (1965).
26. Y. Hayashi, M. Nishizawa, and T. Sakan, Tetrahedron, **33**, 2509 (1977).
27. P. D. Lawley in "Chemical Carcinogens, Second Edition," ed by C. E. Searle; American Chemical Society; Washington, D. C.; 1984; Vol. 1, Chap. 7, pp 325-484.
28. B. Singer and D. Grunberger, "Molecular Biology of Mutagens and Carcinogens," Plenum Press; New York, London; 1983; Chap. IV, pp 45-96.