Table I. Configuration at P_β of ADPβS,β^{18}O Produced by Adenylate Kinase

phosphorylating system	mass % ^{18}O[a]	
	trimethyl phosphate	trimethyl phosphorothioate
acetate kinase	1.1 ± 0.1	83.0 ± 0.2
pyruvate kinase	20.3 ± 0.2	44.6 ± 0.4

[a] The degradation of ATPβS,β^{18}O to trimethyl phosphate and trimethyl phosphorothioate and the mass analysis of those compounds were as described in the preceding paper.[5]

ATPγS is a reasonably good thiophosphoryl donor substrate for adenylate kinase, which catalyzes eq 1:

$$\text{MgATP} + \text{AMP} \rightleftharpoons \text{MgADP} + \text{ADP} \qquad (1)$$

When AMP is thiophosphorylated by **6**, the product is ADPβS,β^{18}O, and the configuration of the β-[^{18}O]phosphorothioate group can be related to that of the γ-[^{18}O]phosphorothioate in **6** by the procedure described in the preceding paper. Thus the configuration at P_γ in compound **6** of this paper is the same as that at P_β in compound **4** and opposite that in compound **5** of the preceding paper.[5] Therefore, if acetate kinase phosphorylates the ^{18}O in ADPβS,β^{18}O produced by adenylate kinase, the configuration is the same as that in **6** (retention) and, if pyruvate kinase phosphorylates this ^{18}O, the configuration is opposite that in **6** (inversion). The data are set forth in Table I which shows that the configuration is opposite that in **6**. We conclude that the reaction occurs with net *inversion* of the configuration of the phosphorthioate group. The least complex interpretation of this result is that the [^{18}O]thiophosphoryl group is transferred directly between the bound donor and acceptor substrates and not via a covalent thiophosphoryl enzyme intermediate.

Our determination of the stereochemical course of adenylate kinase action depends only upon knowledge of the relative configurations of the [^{18}O]phosphorothioate groups prepared in this and the preceding work.[5] The recent assignment of the *S* configuration to P_α of ATPαS isomer A[6] enables us to assign absolute configurations of ATPγS,γ^{18}O and ADPβS,β^{18}O described in this and the preceding paper.

The ^{18}O enrichment in the ADPβS,β^{18}O sample used in Table I was 85.2%. Comparing this with the 83.0% enrichment in trimethyl [^{18}O]phosphorothioate obtained from the ATPβS,β^{18}O sample resulting from acetate kinase catalyzed phosphorylation, it appears that thiophosphoryl group transfer by rabbit muscle adenylate kinase occurs with 97.6% inversion. Given the uncertainties of experimental error and of the magnitude of stereoselectivity exhibited by acetate kinase in the phosphorylation of ADPβS,β^{18}O, this cannot be distinguished from 100% inversion.

Orr et al. have recently prepared ATPγS,γ^{18}O of unknown P_γ configuration and shown that three phosphotransferases catalyze [^{18}O]thiophosphoryl transfer with complete stereospecificity and the same but unknown stereochemical consequences.[7] The present work represents the first synthesis of ATPγS,γ^{18}O with known configuration and the first delineation of the stereochemical course of catalysis by a phosphotransferase.

References and Notes

(1) Supported by Grant GM 24390 from the National Institute of General Medical Sciences.

(2) The abbreviations are ATPαS, adenosine 5′-(1-thiotriphosphate); ADPαS, adenosine 5′-(1-thiodiphosphate); AMPαS, adenosine 5′-phosphorothioate; ATPγS, adenosine 5′-(3-thiotriphosphate).
(3) K.-F. R. Sheu, and P. A. Frey, *J. Biol. Chem.*, **252**, 4445–4448 (1977). AMPαS,α^{18}O was converted to ATPαS,α^{18}O as described and then dephosphorylated to ADPαS,α^{18}O by reaction with excess glucose in the presence of hexokinase.
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(5) J. P. Richard, H.-T. Ho, and P. A. Frey, *J. Am. Chem. Soc.*, preceding paper in this issue.
(6) Personal communications from S. J. Benkovic and F. Eckstein. P. M. J. Burgers and F. Eckstein, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(7) G. A. Orr, J. Simon, S. R. Jones, G. J. Chin, and J. R. Knowles, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2230–2233 (1978).

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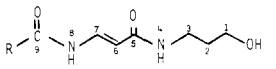
Ultraviolet Chromophores of Palytoxins

Sir:

The palytoxins, exceedingly poisonous substances from marine soft corals of the genus *Palythoa*,[1-3] exhibit ultraviolet absorption maxima at 263 and 233 nm. The λ 263 chromophore of these toxins is associated with a *N*-(3′-hydroxypropyl)-*trans*-3-amidoacrylamide moiety (**1**).[2,4] We report here



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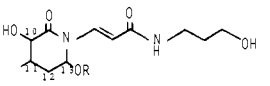
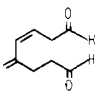
the degradation of palytoxins to **2a** and **5** (isolated and characterized as **6**) which possess the λ 263 and one of the two λ 233 chromophores,[5] respectively.



2a R = H
3a R = Me



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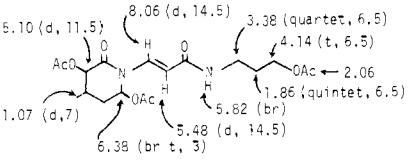
The toxin was oxidized with excess sodium metaperiodate in H_2O at 0 °C and the reaction mixture was extracted with chloroform. The organic material that remained in the aqueous layer was separated by countercurrent distribution (*n*-BuOH, H_2O) to give **2** (λ_{max} 263 nm) as the slowest moving fraction. The ^{1}H NMR spectrum of **2** did not exhibit an aldehydic signal, but **2** was readily converted to **3** when allowed to stand in MeOH-$CHCl_3$ solution. The ^{1}H NMR spectra of **2** and **3** were very similar and both showed doubling of signals for the presence of two closely related compounds.[6,7] The major compounds in **2** and **3** were **2a** and **3a**. Acetylation of **2** and separation of the mixture by TLC on silica gel (20% MeOH-benzene) led to triacetate **4**: UV (EtOH) λ_{max} 257 nm (ϵ 17 300); ^{1}H NMR ($CDCl_3$) 3 H singlets at δ 2.06, 2.09, 2.30. The EI mass spectrum of **4** did not exhibit a molecular ion peak



4

a

but did show small fragment ion peaks at *m/e* 338 and 278 and a very intense peak at *m/e* 134 for successive losses of two HOAc molecules and $CONHCH_2CH_2CH_2OAc$ from the molecular ion[8] to form a (possible structure of *m/e* 134 ion).

The mixture of **5** and other aldehydes in the chloroform fraction was reduced with $NaBH_4$ in 2-propanol and acetylated with acetic anhydride in pyridine. Preparative TLC on silica gel (35% EtOAc-cyclohexane) gave **6**: UV (MeOH) λ_{max} 227 nm; for ^{1}H NMR ($CDCl_3$) see formula; IR ($CHCl_3$) ν_{max} 910 cm^{-1}; mass spectrum m/e (rel intensity) (20 eV), no molecular

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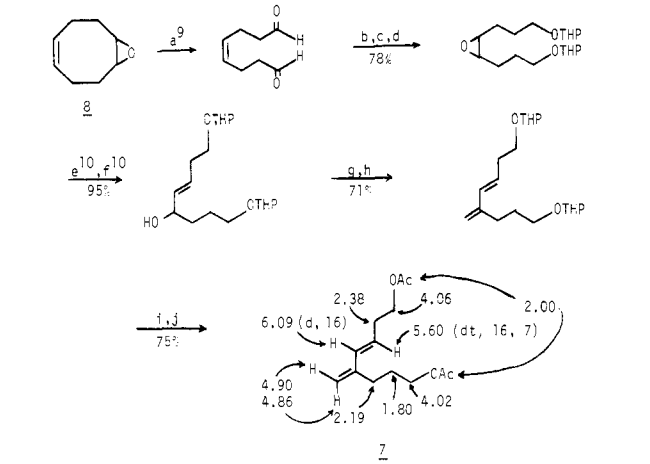
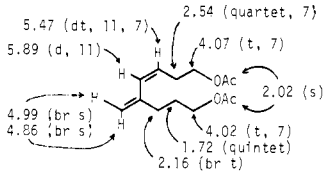
Scheme I[a]

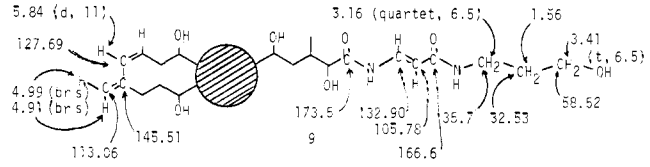


[a] a, H_5IO_6, 45 °C, 2 h; b, $NaBH_4$, EtOH, 0 °C; c, DHP, *p*-TsOH, room temperature, benzene; d, MCPBA, NaOAc, $CHCl_3$, room temperature, 12 h; e, $PhSe^-$, EtOH, Δ, 2 h; f, H_2O_2, room temperature, 6 h; g, DDO, CH_2Cl_2, room temperature, 12 h; h, $Ph_3P{=}CH_2$, THF, Δ. i, H_3O^+, CH_3OH. j, Ac_2O, pyridine.



ion, *m/e* 194 (10, M − CH_2CO − $2H_2$), 180 (10, M − HOAc), 137 (5), 136 (6), 134 (7), 120 (68, M − 2HOAc), 108 (9), 107 (19), 105 (19), 94 (100, M − HOAc − CH_2CHOAc), 92 (27), 79 (16), 67 (3). The corresponding trans isomer **7** was readily synthesized from cyclooctadiene monoepoxide (**8**)[9] as outlined in Scheme I. Photoisomerization of **7** using naphthalene as the sensitizer gave a 1:1.3 mixture of **6** and **7** at the photostationary state which was reacted with maleic anhydride in benzene in a sealed tube (2 h, 90 °C, trace of hydroquinone). Separation of **6** and the Diels-Alder adduct of **7** was achieved by chromatography. The synthetic **6** was identical with **6** from the degraded toxin.

The palytoxins[2] therefore have partial structure **9** (^1H and ^{13}C chemical shifts in Me_2SO-d_6).[11,12]



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Supplementary Material Available: The 90-MHz ^{13}C NMR spectrum of the palytoxin from a Tahitian *Palythoa* sp. in dimethyl sulfoxide-d_6 at 55 °C (4 pages). Ordering information is given on any current masthead page.

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(2) R. E. Moore, R. F. Dietrich, B. Hatton, T. Higa, and P. J. Scheuer, *J. Org. Chem.*, **40**, 540–542 (1975).
(3) Order Zoanthidea, subclass Zoantharia, class Anthozoa, phylum Coelenterata.
(4) The OH on C-1 is free in the palytoxins; i.e., it is not bound in a group such as a ketal. The proton and carbon-13 chemical shifts (in Me_2SO-d_6) of the methylenes in the 3-hydroxypropyl group of the palytoxins are identical with those of *N*-(3′-hydroxypropyl)-*trans*-3-acetamidoacrylamide; also, the C-1 carbon has a longer relaxation time (T_1) than any other proton-bearing carbon in the palytoxins.
(5) ^1H NMR studies of palytoxins at 360 MHz suggest that the second λ 233 chromophore is also a conjugated diene.
(6) ^1H NMR of major compound **3a** (D_2O): δ 7.81 (d, *J* = 14.5, H on C-7), 5.68 (d, *J* = 14.5 Hz, H on C-6), 5.24 (br t, *J* = 3 Hz, H on C-13), 3.68 (d, *J* = 10.5 Hz, H on C-10), 3.44 (t, *J* = 6 Hz, 2 H on C-1), 3.14 (t, *J* = 7 Hz, 2 H on C-3), 3.13 (s, OMe on C-13), 1.57 (quintet, 2 H on C-2), 0.92 (d, *J* = 7 Hz, Me on C-11). ^1H NMR of minor compound **3b** (D_2O): δ 7.49 (d, *J* = 14.5 Hz, H on C-7), 5.72 (d, *J* = 14.5 Hz, H on C-6), 3.13 (s, OMe), 3.12 (t, *J* = 7 Hz, 2 H on C-3), 0.72 (d, *J* = 7 Hz, Me).
(7) The two compounds in **2** are both labile in acid, but decompose at very different rates. In 0.07 N methanolic HCl the λ 263 absorption band disappears with a half-life of 100 h for **2a** and 19 h for **2b**.
(8) Mass spectrum (rel intensity) (20 eV) m/e 338 (1), 278 (8), 162 (2), 134 (100) with metastable ions at *m/e* 228.7 (338 → 278), 64.6 (278 → 134); high resolution mass spectrum *m/e* 338.145 (calcd for $C_{16}H_{22}N_2O_6$, 338.148), 278.124 (calcd for $C_{14}H_{18}N_2O_4$, 278.127), 134.063 (calcd for C_8H_8NO, 134.061).
(9) J. P. Nagarkatti and K. R. Ashley, *Tetrahedron Lett.*, 4599–4600 (1973).
(10) K. B. Sharpless and R. F. Lauer, *J. Am. Chem. Soc.*, **95**, 2697–2699 (1973).
(11) The ^{13}C NMR spectra were determined at 55 °C. Chemical shifts are relative to Me_2SO-d_6 (δ 39.63) as an internal standard. ^{13}C–^1H correlations are based on single-frequency off-resonance decoupling experiments at 90 MHz.
(12) The elemental composition of the palytoxin from Hawaiian *Palythoa toxica* has been roughly estimated to be $C_{145}H_{264}N_4O_{78}$.[1] Its 300-MHz or 360-MHz ^1H NMR spectrum is essentially identical with those of the palytoxins from Jamaican *P. mammilosa* and a Tahitian *Palythoa* sp. The differences are subtle. Minor differences can also be seen in the ^{13}C NMR spectra of these three toxins.[2] Integration of the ^1H NMR spectra suggests that there may be less than 264 protons in the palytoxins. In the proton noise-decoupled ^{13}C NMR spectra of the palytoxins at least 110 carbon peaks can be counted. Integration (under conditions to nullify nuclear Overhauser effects), however, indicates that there may be as many as 145 carbon atoms in the palytoxins.

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Isolation and Characterization of the First Mitotic Cycle Hormone That Regulates Cell Proliferation

Sir:

Since the discovery of a natural substance that regulates cellular proliferation by Bullough and coinvestigators in the early 1960s, numerous investigators have attempted to clarify the chemical structures and physiological activities of various chalones.[1] The importance of these substances as tools in cancer research has become evident.

More recently, we (Evans and Van't Hof)[2] have characterized some physiological parameters of a G2 factor present in cotyledons of peas (*Pisum sativum*) that promotes cell arrest in G2[3] in both roots and shoots after seed germination. Many of the physiological responses of the G2 factor resemble those of chalones. The purpose of this communication is to describe the isolation and characterization of this G2 factor.

Seeds of garden peas (*Pisum sativum*) were surface sterilized and germinated on sterile vermiculite. The cotyledons (~3200) of the 3-day-old seedlings were aseptically excised and incubated in 8 L of sterile distilled water in 160 culture flasks for 2 days. The water extract was then filtered through a graded series of filters until it passed through a 0.30-mesh millipore filter. The extract was evaporated, chromatographed on Dowex 50W-X4,[4] dried, dissolved in 20% ethanol, and chromatographed on Sephadex LH-20.[4] The bioactive fractions which were assayed according to published methods[2] were pooled to give 10 g of residue. Two gel filtrations through Se-

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