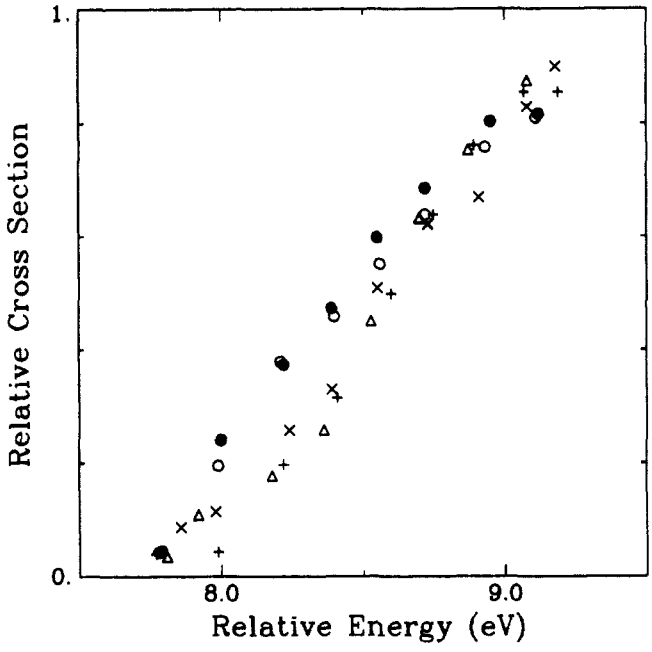

Figure 1. The relative cross section for butyl iodide reacting with tetrakis(dimethylamino)ethylene is plotted against the relative translational energy: (○) *tert*-butyl iodide, (△) *sec*-butyl iodide, (+) *n*-butyl iodide, (×) isobutyl iodide. The open and closed circles are two separate runs. The cross sections were scaled by the following factors: *tert*-butyl iodide, 1.00; *sec*-butyl iodide, 0.44; *n*-butyl iodide, 0.31; and isobutyl iodide, 0.31. The relative cross section is given by the ordinate of the figure times the scaling factor. The butyl iodide beam was held at 26 °C, while the temperature of the TDMAE beam was varied over a range of 40–300 °C to obtain the range of translational energies.

In summary, we have seen the reaction of alkyl iodides with TDMAE, a very strong organic base. The TDMAE abstracts a proton from the alkyl halide which then eliminates I^-. The reaction takes place in a molecular beam and therefore must occur on a single molecular collision. Beyond this, it is not clear what the time scale of the reaction is or whether the elimination occurs immediately during the collision or after the protonated amine has left the vicinity of the reaction. We have seen reaction with isopropyl iodide and with *tert*-butyl bromide with a smaller cross section, so the reaction appears to be more general than with butyl iodides.

The Necessity of an Intact Polyene for the Biological Isomerization of Vitamin A

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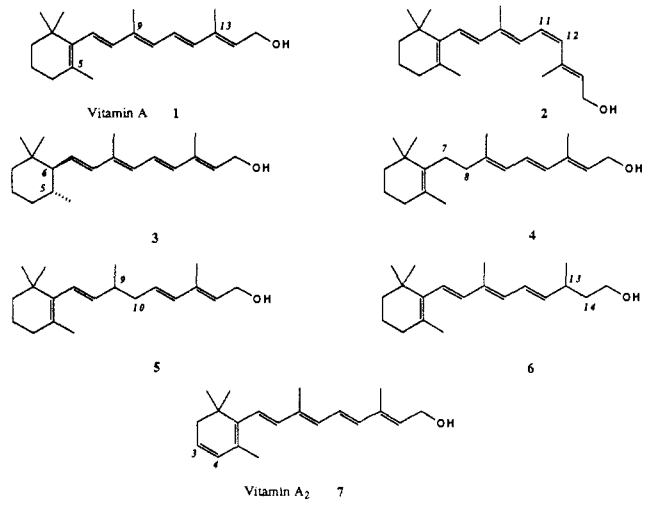
The vertebrate visual cycle is completed by an enzyme system which converts added *all-trans*-retinol (**1**) (vitamin A) into 11-*cis*-retinol.[1-3] The retinol dehydrogenase mediated oxidation of 11-*cis*-retinol to 11-*cis*-retinal provides the chromophore for rhodopsin.[4]

Chart I



The isomerization process is of interest both biologically and chemically—chemically because the isomerization is endothermic, with the product 11-*cis*-retinol being approximately 4.0 kcal/mol less stable than its all-trans congener.[5] Possible mechanisms for the energy requiring isomerization are shown in Scheme I. Here the isomerization event is coupled to the hydrolysis of a putative ester (OX), formed from retinol, which could provide the necessary energy to drive the thermodynamically uphill isomerization.[2] The recent observation that inversion of configuration of the prochiral methylene hydroxyl group of *all-trans*-retinol accompanies isomerization is consistent with the mechanisms shown in Scheme I.[6] Mechanisms of the type shown in Scheme I would predict an important role for the double bonds of the polyene backbone of the substrate in the isomerization process. In this communication, the specificity of the isomerase is probed with respect to dihydro and dehydro-retinol substrates in order to elucidate further the mechanism of action of the enzyme. The experiments show that the polyene system must remain intact in order for appreciable isomerization to occur.

The four dihydroretinols studied are shown below in Chart I. The 5,6-dihydro- (**3**), 7,8-dihydro- (**4**), and 9,10-dihydro-*all-trans*-retinols (**5**) were prepared by $NaBH_4$ reduction of previously reported *trans*-dihydroretinals.[7a] The corresponding 11-cis isomers were obtained similarly, from the 11-cis-dihydro series.[7b] The 13,14-dihydro-*all-trans*-retinol (**6**) was readily prepared by the Wittig reaction between [3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadienyl]triphenylphosphonium bromide and 2-methyl-4-[(*tert*-butyldimethylsilyl)oxy]butanal, followed by deprotection of the silyl ether with tetrabutylammonium fluoride. The 15-^{3}H-labeled dihydro and dehydro *all-trans*-retinols were prepared by the reduction of the corresponding aldehydes with sodium boro[^{3}H]hydride. The label in the 15-position of *all-trans*-retinol is not lost during its isomerization, when subjected to the membrane-bound amphibian or bovine pigment epithelium derived isomerase systems.[3] This membrane fraction also contains retinyl ester synthetase activity, and the latter appears in all membrane fractions containing the isomerase.[2] In addition, unwashed membranes also contain substantial retinol redox activity.[1]

When the retinoids discussed above were subjected to the amphibian isomerase containing membranes (washed and unwashed), they were readily enzymatically esterified to their corresponding palmitate esters, but none were substantially isomerized to their 11-cis congeners (Table I). Small amounts of the 11-*cis*-retinols

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(2) Fulton, B. S.; Rando, R. R. *Biochemistry* **1987**, *26*, 7938-7945.
(3) Bernstein, P. S.; Law, W. C.; Rando, R. R. *J. Biol. Chem.* **1987**, *262*, 16848-16857.
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(5) Rando, R. R.; Chang, A. *J. Am. Chem. Soc.* **1983**, *105*, 2879-2882.
(6) Law, W. C.; Rando, R. R. *Biochemistry* **1988**, *27*, 4147-4152.
(7) (a) Arnaboldi, M.; Motto, M. G.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1979**, *101*, 7082-7084. (b) Koutalos, Y.; Ebrey, T. G.; Tsuda, M.; Park, M. H.; Lien, T.; Derguini, F.; Nakanishi, K. *Biophys. J.*, submitted for publication.

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Scheme I

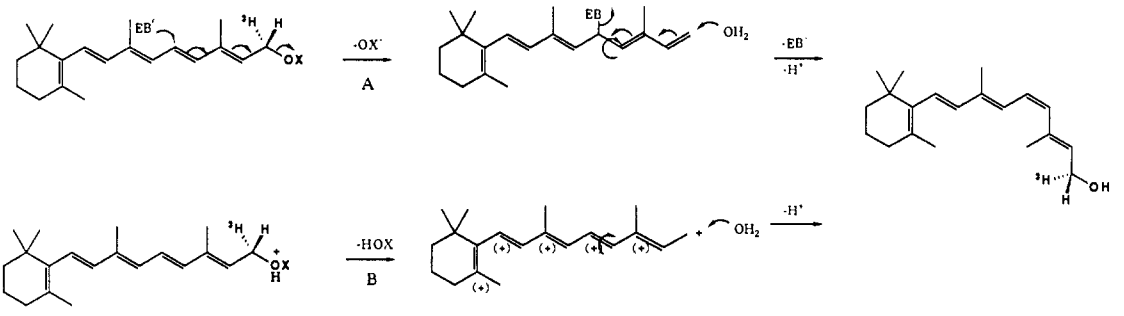

Table I. Incubation of ^{3}H-*all-trans*-Retinol Analogues with Amphibian Retina/Pigment Epithelium Homogenates at Room Temperature for 3 h[a]

substrate	retinol		retinal		retinyl palmitate	
	% 11-cis	% recovery	% 11-cis	% recovery	% 11-cis	% recovery
5,6-DHATOL (**3**)						
without membranes	0.8	>99	/	<1	/	<1
washed membranes (n = 2)	7.1 ± 0.5	8.1 ± 0.7	/[b]		1.3 ± 0.4	91.9 ± 0.7
600 × g super. (n = 2)	5.3 ± 1.0	6.3 ± 2.0	/[c]		1.6 ± 0.4	93.7 ± 2.0
7,8-DHATOL (**4**)						
without membranes	0.2	>99	/	<1	/	<1
washed membranes (n = 2)	2.9 ± 0.8	7.4 ± 0.2	/[b]		0.6 ± 0.4	92.6 ± 0.2
9,10-DHATOL (**5**)						
without membranes (n = 2)	0.7 ± 0.1	>99	/	<1	/	<1
washed membranes (n = 2)	9 ± 5	12 ± 7	/[b]		0.3 ± 0.7	88 ± 7
600 × g super.	7.0	9.3	/[c]		0.3	91
13,14-DHATOL (**6**)						
without membranes (n = 2)	0.3 ± 0.1	>99	/	<1	/	<1
washed membranes (n = 2)	0.9 ± 0.1	10.0 ± 1.4	/[b]		0.0[d]	87
vitamin A_2 (**7**)						
without membranes (n = 3)	0.1 ± 0.1	>99	/	<1	/	<1
washed membranes (n = 3)	40.3 ± 8.9	16.2 ± 0.9	/[b]		33.4 ± 17.6	83.8 ± 0.9
600 × g super. (n = 3)	11.5 ± 4.6	45.6 ± 15.1	70.4 ± 2.3	8.6 ± 2.1	20.5 ± 4.2	45.8 ± 14.0
all-trans-retinol (**1**)						
without membranes	0.3	>99	/	<1	/	<1
washed membranes	43.9	15.0	/[b]		21.1	85.0
600 × g super.	24.1	16.5	57.5	9.0	18.1	74.5

[a] The preparation of amphibian retina/pigment epithelium 600 × g supernatant of washed membranes was described elsewhere;[1] the 600 × g supernatant has retinol redox activity and ester synthetase activity along with the retinol isomerase activity, while in the washed membranes the redox activity is reduced, owing to the removal of the soluble pyridine nucleotide coenzymes. For a typical assay, 0.2–0.5 μCi of the 15-^{3}H-labeled substrate was incubated with 300 μL of membranes and 15 μL of 10% BSA (as retinol carrier) at room temperature for 3 h; the retinoids were extracted by a standard procedure.[1] A control containing no eye tissue (without membranes), but otherwise identical, was prepared for each assay. [b] Not determined. [c] No significant formation of retinyl oximes was observed. [d] The palmitate isomers were not separated by HPLC. Therefore, the palmitates formed from the incubation were collected by HPLC and hydrolyzed with 5% KOH/MeOH at 0 °C to form the corresponding alcohols, and their isomeric composition was analyzed by standard methods.[1] DHATOL refers to dihydro-*all-trans*-retinol.

appeared to be formed, but it must be remembered that this conversion accounts for less than 1% of the total radioactive *all-trans*-retinol added and may not be significant. This view can be further supported by examining the retinyl palmitate pool. Substantial amounts of 11-*cis*-retinyl palmitate are found when vitamin A is the substrate[1] (Table I). The 11-*cis*-retinyl palmitate probably arises by direct esterification of 11-*cis*-retinol. With the dihydroretinols as substrates, no significant amounts of 11-*cis*-retinyl palmitates were found. For the sake of comparison, data are also shown for the natural substrate *all-trans*-retinol where substantial isomerization is observed. These results demonstrate (1) the high level of specificity inherent in the isomerization process and (2) that an intact polyene system be present in order for appreciable isomerization to ensue. The results obtained in the amphibian isomerase system were repeated with the isomerase found in the bovine pigment epithelium, with similar results (Table II).

Given the above findings, it was of interest to study 3,4-dihydro-*all-trans*-retinol[8] (**7**) (vitamin A_2) as a possible substrate, since the polyene conjugation of retinol is further extended here. As shown in Table I, this dehydroretinol is an excellent substrate for the amphibian enzyme and is substantially processed to 11-*cis*-retinol, 11-*cis*-retinal, and 11-*cis*-retinyl palmitate. The bovine enzyme was also readily able to isomerize vitamin A_2 (Table II). It is of interest to note that early in development in the amphibian,

(8) Henbest, H. B.; Jones, E. R. H.; Owen, T. C.; Thaller, V. *J. Chem. Soc.* **1955**, 2763–2767.

Table II. Incubation of ^{3}H-*all-trans*-Retinol Analogues with Bovine Pigment Epithelium Washed Homogenates at 37 °C for 1 h[a]

substrate	retinol % 11-cis	retinol % recovery	retinyl palmitate % 11-cis	retinyl palmitate % recovery
5,6-DHATOL (3)				
without membranes	0.6	>99	/	<1
with membranes	3.7	19.8	1.2	80.2
7,8-DHATOL (4)				
without membranes	0.2	>99	/	<1
with membranes (n = 2)	3.9 ± 2.2	6.1 ± 2.6	0.6 ± 0.1	93.9 ± 2.6
9,10-DHATOL (5)				
without membranes	1.4	>99	/	<1
with membranes	4.3	30.5	2.5	69.5
vitamin A_2 (7)				
without membranes	0.1	>99	/	<1
with membranes (n = 2)	69.2 ± 1.2	18.3 ± 0.9	27.7 ± 3.9	81.7 ± 0.9
all-trans-retinol (1)				
without membranes	0.3	>99	/	<1
with membranes	52.1	26.5	25.1	73.5

[a] The preparation of bovine pigment epithelium washed membranes is described elsewhere;[2] 0.2 μCi of the 15-^{3}H-labeled substrate was incubated with 300 μL of the bovine pigment epithelium washed membranes and 15 μL of 10% BSA (as retinol carrier) for 1 h at 37 °C. The retinoids were extracted and analyzed by standard methods.[1] The formation of retinal is not significant in this membrane preparation. DHATOL refers to dihydro-*all-trans*-retinol.

the rhodopsin (porphyropsin) is based on the vitamin A_2 system.[9]

Acknowledgment. This work was supported by United States Public Health Service Research Grants EY 04096 and GM 36564 from the National Institutes of Health.

(9) Knowles, A.; Dartnall, H. J. A. In *The Eye*; Davson, H., Ed.; Academic Press: New York, 1977; Vol. 2B, Chapter 12.

A Highly Efficient, Practical Approach to Natural Taxol†

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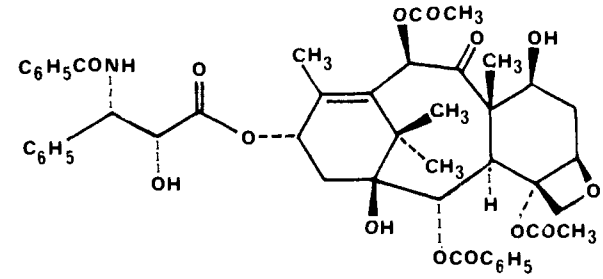
Taxol (**1**)[1] is an exceptionally promising cancer chemotherapeutic agent with an unusually broad spectrum of potent antileukemic and tumor-inhibiting activity.[2] Taxol is active in vivo against P-388, P-1534, and L-1210 mouse leukemias, B-16 melanocarcinoma, Lewis lung carcinoma, sarcoma 180, and CX-1 colon, LX-1 lung, and MX-1 breast xenographs.[1-3] The bark



Taxol (1)

from several species[4] of yew (genus *Taxus*, family Taxaceae), very slow-growing evergreens,[5] currently supplies taxol; the isolation procedure, however, is difficult, low-yielding,[6] and, obviously, fatal to the source, which is threatened. There is widespread concern that, ironically, "if taxol proves effective...the yew population could be so severely depleted that there would not be enough trees left to make treatment successful"[7] and that "alternative sources will need to be found to cater for the increased demand".[8] The National Cancer Institute has recently contracted for 27 000 kg of yew bark.[8]

The structural novelty of this complex, highly functionalized diterpene together with its exciting therapeutic potential has engendered worldwide a prodigious effort toward its total synthesis.[9] Taxol is quite possibly the number one target today of synthetic organic chemists. The various strategies revealed to date appear, however, to be of little practical value in that even if successful they would probably be incapable of furnishing the natural product in more than trace amounts.

In contrast, an efficient partial synthesis of taxol from an easily and permanently accessible taxol congener would provide an attractive solution to this serious supply problem. We report in this communication a direct synthesis of taxol through the successful implementation of such an approach.

10-Deacetyl baccatin III (**2**, Scheme I) can be readily extracted in high yield from the leaves of *Taxus baccata* L.[10] It is important to recognize that the yew leaves are quickly regenerated, hence through prudent harvesting large amounts of **2** can be continually

† Dedicated to the memory of Professor Pierre Crabbé.

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325-2327.

(2) Taxol (NSC-125973) is currently in phase II clinical trials in the United States. It is the only plant product known to promote the assembly of microtubules and inhibit the tubulin disassembly process and, thus, appears to be the prototype of a new class of cancer chemotherapeutic agents. See: Suffness, M.; Cordell, G. A. In *The Alkaloids, Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: Orlando, FL, 1985; Vol. XXV, Chapter 1.

(3) Douros, J.; Suffness, M. In *Recent Results in Cancer Research*; Carter, S. K., Sakurai, Y., Umezawa, H., Eds.; Spinger-Verlag: Berlin, 1981; Vol. 76, pp 169-170. Lomax, N. R.; Narayanan, V. L. *Chemical Structures of Interest to the Division of Cancer Treatment*; U.S. Government Printing Office: Washington, D.C., 1983; Vol. III, p 17. Engel, S. I.; Schwartz, E. L.; Strauman, J. J.; Wiernik, P. H. *Proc. Am. Assoc. Cancer Res.* **1985**, *26*, 158. Zee-Cheng, R. K.-Y.; Cheng, C. C. *Drugs of the Future* **1986**, *11*, 45-48.

(4) (a) Miller, R. W.; Powell, R. G.; Smith, C. R., Jr.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1981**, *46*, 1469-1474. (b) Sénilh, V.; Blechert, S.; Colin, M.; Guénard, D.; Picot, F.; Potier, P.; Varenne, P. *J. Nat. Prod.* **1984**, *47*, 131-137. (c) Magri, N. F.; Kingston, D. G. I. *J. Org. Chem.* **1986**, *51*, 797-802. (d) See, also: Sénilh, V. Ph.D. Dissertation, Université de Paris-Sud, Orsay, 1984, and references cited therein.

(5) The yew is one of the slowest growing trees in the world, growing at less than one-tenth the rate of the Douglas fir.[7]

(6) The reported yields of taxol from various species of yew range from 40 to 165 mg/kg.[4]

(7) New York Times, May 3, 1987, p 29.

(8) Suffness, M. National Cancer Institute, personal communication.

(9) For a compilation of references through 1986, see: Berkowitz, W. F.; Amarasekara, A. S.; Perumattam, J. J. *J. Org. Chem.* **1987**, *52*, 1119-1124. For more recent work, see: Swindell, C. S.; Patel, B. P.; deSolms, S. J. *J. Org. Chem.* **1987**, *52*, 2346-2355. Hua, D. H.; Gung, W.-Y.; Ostrander, R. A.; Takusagawa, F. *J. Org. Chem.* **1987**, *52*, 2509-2517. Lin, J.; Nikaido, M. M.; Clark, G. *J. Org. Chem.* **1987**, *52*, 3745-3752. Wender, P. A.; Snapper, M. L. *Tetrahedron Lett.* **1987**, *28*, 2221-2224. Pettersson, L.; Frejd, T.; Magnusson, G. *Tetrahedron Lett.* **1987**, *28*, 2753-2756. Swindell, C. S.; Patel, B. P. *Tetrahedron Lett.* **1987**, *28*, 5275-5278. Bonnert, R. V.; Jenkins, P. R. *J. Chem. Soc., Chem. Commun.* **1987**, 1540-1541. Shea, K. J.; Haffner, C. D. *Tetrahedron Lett.* **1988**, *29*, 1367-1370. Trost, B. M.; Fray, M. J. *Tetrahedron Lett.* **1988**, *29*, 2163-2166.

(10) Chauvière, G.; Guénard, D.; Picot, F.; Sénilh, V.; Potier, P. *C. R. Seances Acad. Sci., Ser 2* **1981**, *293*, 501-503. We are currently obtaining **2** in yields of ca. 1 g/kg of fresh leaves. (It should be noted that **2** is far less active than taxol.[1]) For previous transformations of **2**, including an alternative approach to taxol, see references 12a,b and Colin, M.; Guénard, D.; Guéritte-Voegelein, F.; Potier, P. French Patent 2 601 676, 1986.

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