NOTES

PREPARATION OF 11-CIS-RETINAL-11-^{3}H

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SUMMARY

trans-Retinal-11-^{3}H was photolyzed by a fluorescent source to afford a mixture of isomeric retinals, from which 11-cis-retinal-11-^{3}H was isolated by HPLC

Key Words: 11-cis-Retinal-11-^{3}H, photolyses, all-trans-retinal-11-^{3}H

INTRODUCTION AND DISCUSSION

11-cis-Retinal, a Vitamin A aldehyde known to form photosensitive pigments necessary for vision has been prepared labeled with tritium by a photochemical procedure starting with tritiated all-trans-retinal.

Brown and Wald first prepared 11-cis-retinal in a tedious way from all-trans-retinal by exposing it to sunlight.[1] Recently, papers have appeared on the simultaneous determination of retinal (and other retinoids) isomers by high-performance liquid chromatography (HPLC)[2,3,4] and on the adaptation of this technique to the preparation of 11-cis-retinal[5,6] and 7-cis-retinal[3] by photochemical means.

The irradiation of all-trans-retinal was done in ordinary glassware at room temperature and under a blanket of argon with two circular fluorescent lamps. Acetonitrile was used as a solvent, because it was found that the highest yields of 11-cis-retinal were obtained in that solvent.[3,6] The reaction was followed by HPLC. Figure 1 shows the isomer mixture after 2

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HIGH-PERFORMANCE LIQUID CHROMATOGRAM OF A MIXTURE OF ^{3}H-RETINAL ISOMERS OBTAINED BY IRRADIATION OF ALL-TRANS-11-^{3}H-RETINAL FOR 2.5 HOURS IN ACETONITRILE

WATERS RADIAL PAK B [8SIIOμ]
HEXANE/ETHYL ETHER 88/12, 2 ml/min
UV DETECTION AT 325 nm.

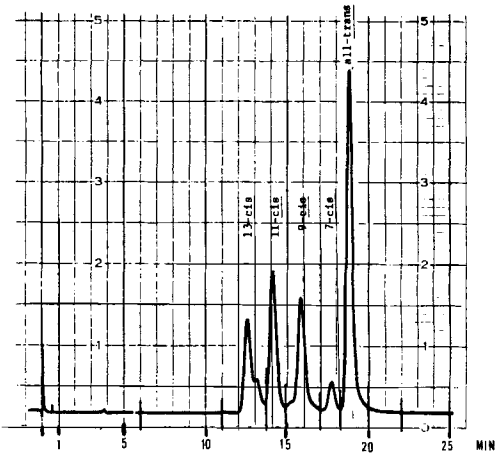

Figure 1

hours of irradiation and Table 1 below shows the distribution of isomers measured by determination of radioactivity in appropriate collected HPLC fractions.

Using HPLC, it was also found that irradiation for periods longer than 2.5 hours did not produce more of the wanted 11-cis-retinal. The peak-heights indicated that there was no increase in any of the isomers, just a constant decrease of all-trans-retinal. The irradiation was therefore terminated after 2.5 hours. The reaction mixture was then concentrated and the products were separated by HPLC. As shown in Table 1 13-cis, 11-cis, 9-cis and all-trans-retinals were isolated and identified by spectroscopic methods as major components of the mixture when applied onto the HPLC column dissolved in hexane/ether. Application of the reaction mixture dissolved in acetonitrile onto the column resulted in only partial resolution.

Table 1

ISOMERS	AMOUNT PRESENT (%)[2] BY HPLC[1]		AMOUNT ISOLATED (%)[3] BY HPLC[1]		
	AT START	AFTER 2 HR IRRADIATION	4	5	6
13-cis-11-^{3}H-RETINAL	10.1	19.9	13.8	10.6	9.6
11-cis-11-^{3}H-RETINAL	--	24.2	13	11.2 (11.7)[7]	(6.4)[7]
9-cis-11-^{3}H-RETINAL	--	18.3	NOT RESOLVED	7.2	(5.1)[7]
7-cis-11-^{3}H-RETINAL	--	4.5		--	--
ALL-TRANS-11-^{3}H-RETINAL	86.4	29.9		13.7	12.8

1. WATERS RADIAL PACK B [8SI10μ], HEXANE/ETHYL ETHER 88/12.
2. IN % OF TOTAL ELUATE.
3. OVERALL RADIOCHEMICAL YIELD.
4. INJECTED IN SMALL INCREMENTS DISSOLVED IN ACETONITRILE.
5. INJECTED IN SMALL INCREMENTS DISSOLVED IN HEXANE/ETHYL ETHER 88/12.
6. A PARTISIL 10 COLUMN, [50 CM X 1 CM], WAS USED FOR THE SEPARATION. INJECTION OF LARGE INCREMENTS OF SAMPLE DISSOLVED IN HEXANE ETHER 88/12.
7. FIGURES IN BRACKETS INDICATE THAT COMPOUND WAS OBTAINED WITH A RADIOPURITY LOWER THAN 96%. ONE MORE PASSAGE THROUGH THE HPLC USUALLY YIELDED A PRODUCT WITH ACCEPTABLE RADIOPURITY.

11-cis-Retinal-11-^{3}H was stored as a solution in toluene and kept in the dark at -50°C. As shown in Table 2 it was quite stable, but rearranged to some extent to all-trans- and 13-cis-retinal. Storage at -60°C seems to increase the stability.

Table 2

STORAGE STABILITY DATA FOR 11-cis-11-^{3}H-RETINAL (HPLC PURIFIED)

Specific Activity (Ci/mmol)	Conc. (mCi/ml)	Activity Stored (mCi)	Storage Time (days)	Storage Temp. (°C)	Composition			% Purity Total Retinal Content
					11-cis	all-trans	13-cis	
1.88					97.1	0.5	2	97
1.88	0.78	7.8	25	-50	96.2	1.3	1.9	97
1.88	0.78	7.8	431	-50	83.5	7.3	5.8	90.8
1.75					95	4		98
1.75	0.5	1	71	-60	95	3	1	98
1.75					97.4	2.2		99
1.75	0.5	1	71	-60	94.5	4.0		98

EXPERIMENTAL

Radioassays were carried out in 10 ml of Scintisol cocktail (Isolab Inc.) with internal standards and counted with a Beckman LS-250 liquid scintillation system. Analyses by HPLC were obtained from a Waters 6000A solvent delivery system, U6K injector, Model 450 variable wavelength detector and a RCM-100 radial compression module using a Radial-Pak B (8Si10μ; 8 mm ID x 10 cm). Unless otherwise noted, analyses were done with hexane/ethylether 88/12 at 2 ml/min with detection at 280 and 325 nm. A sample of 11-cis-retinal was obtained from Hoffmann-LaRoche Inc., Nutley, NJ and 13-cis-retinal was prepared from all-trans-retinal.[7]

Preparation of 11-cis-11-^{3}H-Retinal

Retinal-11-^{3}H (8.7 mg, 1.96 Ci/mmol, 60 mCi)--consisting of a mixture of 86% all-trans and 10% 13-cis isomers--was dissolved in 10 ml of acetonitrile, stirred at room temperature under a blanket of argon and irradiated with two circular fluorescent lamps (Sylvania FC 12T10-CW-RS, 34 watts). The reaction was monitored by HPLC. After 150 min of irradiation, the solvent was removed in vacuo at room temperature and the residue redissolved in 1 ml of hexane/diethyl ether 88/12. Aliquots of 75 μl of this solution were then injected onto a Radial-Pak B column (8Si10μ) for HPLC separation. The products were eluted with 12% diethyl ether in hexane (1 ml/min), with UV detection at 265 nm and 2 AUFS. Appropriate fractions were collected with light excluded. The fractions containing the wanted 11-cis-11-^{3}H-retinal were combined, evaporated to dryness in vacuo without the use of heat, taken up in 10 ml of toluene, and stored at -50°C. This way 7.2 mCi of tritiated 11-cis-retinal with a radiochemical purity of 90% was obtained. Purification of this material by rechromatographing by HPLC gave 6.7 mCi 11-cis-retinal-11-^{3}H (11.2% overall yield; UV λ_{max} 373, shoulder 315,260) with a radiochemical purity of 95% (containing in addition 4% all-trans-retinal). Under the described HPLC conditions, 6.4 mCi of 13-cis-retinal-11-^{3}H (95% radiopurity) and 8.2 mCi all-trans--retinal-11-^{3}H (97% radiopurity) were also cleanly separated.

In a separate identical run the residue after evaporation was redissolved in 0.5 ml acetonitrile. Aliquots of 40 µl of this solution were then injected onto a HPLC column. This way 7.8 mCi (13% overall yield, 97% radiopurity) 11-cis-retinal-11-^{3}H and 8.3 mCi (96.4% radiopurity) 13-cis-retinal-11-^{3}H were obtained, but the all-trans- and the 9-cis-isomers did not resolve under the conditions used.

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