TABLE II

BIOPOTENCIES OF GEOMETRIC ISOMERS OF VITAMIN A ACETATE

Isomer	Bioassays	Mean CF	$E^{1\%}_{1\,cm.}$ [6]	Biopotency, units/g.	Relative biopotency, %
All-*trans*	By definition	(1900)	(1530)	(2,907,000)	(100)
Neo (2-mono-*cis*)	13	1523	1435	2,190,000	75.3
6-Mono-*cis*	3	528	1200	634,000	21.8
2,6-Di-*cis*	3	619	1112	688,000	23.7
			Mean "6-*cis*"	661,000	22.7
2,4-Di-*cis*	3	715	950 (est.)	679,000	23.4

cis-vitamin A acetates appear on the basis of both structure and biopotency to constitute a "6-*cis*" class of vitamin A isomers. The rat can utilize the "6-*cis*"-vitamin A acetates equally well for both growth and for liver storage but only about 23% as well as the all-*trans* isomer. Chick bioassays have resulted in a similarly low bioactivity for the "6-*cis*" isomeric acetates.[13] Since both the rat and the chick respond poorly to the "6-*cis*" isomers and the rat uses the 2,4-di-*cis* isomer poorly, it is probable that humans would have similar difficulty in efficiently utilizing vitamin A other than the all-*trans* and neo isomers.

Vitamin A isomers of low biological activity may occur naturally in certain instances. Fisher, Kon and Thompson[16] have reported on the occurrence of vitamin A in certain marine Crustacea. The physicochemical potencies of such concentrates were reported to be 2–3 times higher than their biological potencies. A few sources of natural vitamin A have been observed in these laboratories to possess physicochemical potencies substantially in excess of their biological activities. Such discrepancies can be accounted for by the presence of isomers other than all-*trans* and neo-vitamin A.

From a practical point of view, the presence of the "6-*cis*" or 2,4-di-*cis* isomers in vitamin A preparations would be difficult to detect by physical and chemical assays. The use of a bioassay, either growth or liver storage, is the best procedure available at present for readily indicating the presence of significant amounts of these isomers. The use of a biological assay is recommended for the evaluation of natural or synthetic vitamin A concentrates of questionable composition.

Acknowledgment is made to N. D. Embree and R. W. Lehman for advice, to H. A. Risley for the performance of vitamin A analyses of livers, to the Manufacturing Control Laboratory for spectrophotometric analyses, and to the Organic Chemistry Department for furnishing the pure vitamin A compounds.

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(16) L. R. Fisher, S. K. Kon and S. Y. Thompson, *J. Marine Biol. Assoc. United Kingdom*, **31**, 229 (1952).

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Biochemical Studies on Vitamin A. XV. Biopotencies of Geometric Isomers of Vitamin A Aldehyde in the Rat[1]

BY STANLEY R. AMES, WILLIAM J. SWANSON AND PHILIP L. HARRIS

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The physiological potencies of five geometric isomers (all-*trans*, neo (2-mono-*cis*), 6-mono-*cis*, 2,6-di-*cis*, and 2,4-di-*cis*) of vitamin A aldehyde and of α-vitamin A aldehyde have been determined. All-*trans* and neo-(2-mono-*cis*)-vitamin A aldehydes have the same biopotency of 3,070,000 u./g., about 91% the molar bioactivity of all-*trans*-vitamin A acetate. The 6-mono-*cis*- and 2,6-di-*cis*-vitamin A aldehydes have the relatively low biopotencies of 637,000 and 581,000 u./g., respectively, about 18% the molar bioactivity of all-*trans*-vitamin A acetate. The 2,4-di-*cis*-vitamin A aldehyde has a biopotency of 1,610,000 u./g., about 48% the molar bioactivity of all-*trans*-vitamin A acetate. The α-ionone analog of vitamin A aldehyde has less than 2% the potency of vitamin A acetate and during metabolism it is converted to the corresponding α-vitamin A alcohol which is stored in the liver.

Five crystalline geometric isomers of vitamin A aldehyde have now been characterized chemically and physically. Crystalline all-*trans*-vitamin A aldehyde[2–4] was reported by Wendler *et al.*[5] to be substantially as active as vitamin A in the growth test. Ames, *et al.*,[6] have recently reported both the all-*trans*- and neo-(2-mono-*cis*)-vitamin A aldehydes[3,4] to have biopotencies of about 3,000,000 u./g. The "*cis*-vitamin A aldehyde" of Graham, *et al.*,[7] was reported to be as active as vitamin A. An isomer with similar chemical properties has since been characterized as the 6-mono-*cis* aldehyde.[4] The 2,6-di-*cis*-vitamin A aldehyde was recently synthesized and crystallized by Robeson,

(1) Presented in part before the Division of Biological Chemistry at the 126th Meeting of the American Chemical Society, New York, New York, September, 1954.

(2) S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J.*, **42**, 516 (1948).

(3) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 269 (1952–53).

(4) C. D. Robeson, W. P. Blum, J. M. Dieterle, J. D. Cawley and J. G. Baxter, THIS JOURNAL, **77**, 4120 (1955).

(5) N. L. Wendler, C. Rosenblum and M. Tishler, *ibid.*, **72**, 234 (1950).

(6) S. R. Ames, W. J. Swanson, H. A. Risley and P. L. Harris, *Federation Proc.*, **13**, 174 (1954).

(7) W. Graham, D. A. VanDorp and J. F. Arens, *Rec. trav. chim.*, **68**, 609 (1949).

et al.[4] The neoretinene-b of Hubbard and Wald[3] was recently crystallized in larger amounts by Dieterle and Robeson[8] and its structure indicated by Robeson, *et al.*,[4] to be 2,4-di-*cis*-vitamin A aldehyde.

The α-ionone analog of vitamin A aldehyde (α-vitamin A aldehyde) has been recently synthesized and crystallized by Robeson, *et al.*[4]

The present paper summarizes the rat bioassay data relevant to the five geometric isomers of vitamin A aldehyde and to α-vitamin A aldehyde.

Experimental

The crystalline vitamin A aldehydes used in this investigation were synthesized as recently described.[4,8] Both rat growth and rat liver storage bioassays were run, as previously described,[9] on the isomeric aldehydes using the USP Reference Standard as the reference material.

Results

The results of both rat growth and rat liver storage bioassays of the geometric isomers of vitamin A aldehyde compared with the USP Vitamin A Reference Standard are given in Table I. The average conversion factor determined for each isomer has been multiplied by the specific absorbancy at the ultraviolet maximum in order to give the best estimate of the biopotency of the specific isomer. The average biopotencies were calculated and the results are shown in Table II. Since all-*trans*-vitamin A acetate has a defined biopotency of 2,907,000 u./g.,[10,11] the isomeric aldehydes, if they were fully active on a molar basis, would have a biopotency of 3,357,000 u./g.

Based on both growth and liver storage bioassays, all-*trans*-vitamin A aldehyde has a mean conversion factor of 1991 and a biopotency of 3,050,000 u./g. Good agreement was observed between growth and liver storage bioassays. Neo-(2-mono-*cis*)-vitamin A aldehyde has a mean conversion factor of 2497 and a biopotency of 3,120,000 u./g. These two "4,6-di-*trans*" isomers of vitamin A aldehyde have very similar activities with an average biopotency of 3,070,000 u./g., about 91% of the bioactivity of all-*trans*-vitamin A on a molar basis.

The bioassays of the 6-mono-*cis*-vitamin A aldehyde gave a mean conversion factor of 524 and a biopotency of 637,000 u./g. The conversion factor of the 2,6-di-*cis* isomeric aldehyde was 510 and the biopotency was 581,000 u./g. The two "6-*cis*" isomers of vitamin A aldehyde have very similar activities with an average biopotency of 615,000 u./g., about 18% that of all-*trans*-vitamin A acetate on a molar basis. These results are at variance with the previous report[7] that a "*cis*-aldehyde" was as active as vitamin A.

The 2,4-di-*cis*-vitamin A aldehyde (neoretinene-b) was bioassayed both as a purified concentrate and as crystalline material. The results were similar on both preparations yielding a mean conversion factor of 1874 and a biopotency of 1,610,000 u./g., about 48% of the molar activity of all-*trans*-vitamin A acetate.

The α-ionone analog of vitamin A aldehyde was examined by both growth and liver storage bioassays. This isomer did not support growth at the lowest level tested and thus has a biopotency less than 50,000 u./g. In the liver storage type of bioassay there was no detectable vitamin A in the liver. By both rat bioassay procedures, α-vitamin A aldehyde is essentially inactive. Examination of the liver oil revealed the presence of α-vitamin A alcohol. This was isolated, characterized and determined spectrophotometrically by its characteristic ultraviolet absorption.[4] About 39% of the ingested dose of aldehyde was found in the liver as alcohol. Since about 65% of an ingested dose of all-*trans*-vitamin A acetate is found in the liver, the relative efficiency of liver storage of α-

TABLE I

BIOASSAYS OF GEOMETRIC ISOMERS OF VITAMIN A ALDEHYDE

(Bioassay potency in terms of USP Reference Standard.)

Type of bioassay	Bioassay potency, u./g.	$E^{1\%}_{1\,cm.}$	Conversion factor
All-*trans*-vitamin A aldehyde		λ_{max} 381 mμ	
LS	3,090,000	1437	2138
LS	2,970,000	1510	1964
LS	3,080,000	1530	2010
LS	3,070,000	1545	1987
LS	2,790,000	1545	1808
G	2,140,000	1170	1826
LS	2,970,000	1530	1938
G	3,490,000	1545	2259
			Mean = 1991 ± 54 S.E.
Neo-(2-mono-*cis*)-vitamin A aldehyde		λ_{max} 375 mμ	
LS	3,480,000	1225	2841
LS	2,940,000	1225	2402
LS	2,730,000	1215	2249
			Mean = 2497
6-Mono-*cis*-vitamin A aldehyde		λ_{max} 373 mμ	
LS	608,000	1215	500
LS	649,000	1215	534
G	658,000	1224	538
			Mean = 524
2,6-Di-*cis*-vitamin A aldehyde		λ_{max} 368 mμ	
LS	610,000	1120	545
LS	532,000	1120	475
			Mean = 510
2,4-Di-*cis*-vitamin A aldehyde		λ_{max} 376 mμ	
LS	1,830,000	870	2106
LS	1,550,000	855	1814
LS	1,530,000	857	1785
LS	1,380,000	857	1610
G	1,760,000	857	2054
			Mean = 1874

(8) J. M. Dieterle and C. D. Robeson, *Science*, **120**, 219 (1954).

(9) S. R. Ames, W. J. Swanson and P. L. Harris, THIS JOURNAL, **77**, 4134 (1955).

(10) Pharmacopeia of the United States of Amerita, "U.S.P. Vitamin A Reference Standard, Instructions for Use," May 18, 1948.

(11) *World Health Organization Technical Report Series*, **3**, 4 (1950).

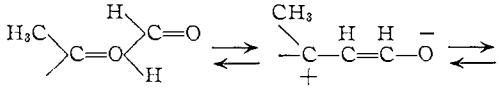
TABLE II
BIOPOTENCIES OF GEOMETRIC ISOMERS OF VITAMIN A ALDEHYDE

Isomer	No. of bioassays	Mean CF	$E^{1\%}_{1\text{ cm.}}$ [4]	Biopotency, units/g.	Relative molar biopotency, % (terms of acetate) 3,357,000 u./g. = 100%	Relative molar biopotency, % (terms of *trans* aldehyde) 3,050,000 u./g. = 100%
All-*trans*	8	1991	1530	3,050,000	90.9	(100)
Neo-(2-mono-*cis*)	3	2497	1250	3,120,000	92.9	102.3
			Mean "4,6-di-*trans*"	3,070,000		
6-Mono-*cis*	3	524	1215	637,000	19.0	20.9
2,6-Di-*cis*	2	510	1140	581,000	17.3	19.0
			Mean "6-*cis*"	615,000		
2,4-Di-*cis*	5	1874	857	1,610,000	48.0	52.8

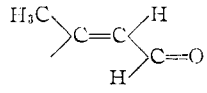
vitamin A aldehyde was about 60%. This estimate is probably low since purification steps preceded the spectrophotometric determination. Thus although biologically inactive as vitamin A, the α-ionone analog of vitamin A aldehyde is reduced, absorbed, transported and stored like vitamin A aldehyde itself.

Discussion

As a result of this investigation and the data reported in an accompanying publication[9] the biopotencies of both the aldehyde and acetate forms of the five geometric isomers of vitamin A have been determined. Neo-(2-mono-*cis*)-vitamin A acetate has been previously shown[12] to be about 25% less active than the all-*trans* isomeric acetate. However, the all-*trans* and neovitamin A aldehydes have identical biopotencies at about 91% of the all-*trans* acetate on a molar basis. These biopotency relationships are explainable by postulating that during the digestive process these two isomeric aldehydes can be interconverted as



2-*trans* aldehyde



2-*cis* aldehyde

Since the carbonyl is in conjugation with the 2-double bond such an equilibrium can be established[13] between the 2-*cis* and 2-*trans* aldehydes but not between the 2-*cis* and 2-*trans* acetates. Since neovitamin A alcohol has the same biopotency as the corresponding acetate,[12] this equilibrium between the 2-mono-*cis* and all-*trans* aldehydes must be established prior to reduction. It probably takes place prior to the absorption of vitamin A through the intestinal wall since no vitamin A aldehyde can be detected in the blood or in the liver.[6] Following absorption the *in vivo* interconversion of the all-*trans* and *neo* forms of vitamin A probably proceeds as previously described.[12]

An even greater difference in relative molar biopotency exists between the 2,4-di-*cis*-vitamin A aldehyde and the corresponding acetate. The biopotency of the aldehyde is 205% that of the acetate which is additional evidence in support of the 2-*cis* configuration of the 2,4-di-*cis* structure assigned by Robeson, *et al.*[4]

No such difference in relative molar biopotency exists in the case of the 6-mono-*cis* and 2,6-di-*cis* isomers of vitamin A aldehyde and acetate. There is probably *cis–trans* interconversion of the aldehydes about the 2-double bond, but because both 6-*cis* acetates have the same biopotency, this interconversion of the 2-double bond would not affect the biological response.

The biological action of the five geometric isomers of vitamin A aldehyde in an *in vitro* rhodopsin system has been discussed by Hubbard and Wald.[3] In their system in the absence of light the 2,4-di-*cis*-vitamin A aldehyde (neoretinene-b) was quite specific as the precursor of rhodopsin. The 6-mono-*cis* isomer (isoretinene-a) yielded a light-sensitive pigment, isorhodopsin, with a displaced absorption spectrum. The all-*trans*, neo (neoretinene-a) and 2,6-di-*cis*-(isoretinene-b)-vitamin A aldehydes did not form appreciable quantities of light sensitive pigments. Thus, there is no correlation between the biological potencies of the geometric isomers of vitamin A aldehyde as determined by rat bioassay and their action in the *in vitro* rhodopsin system.

In order to rationalize the existence of five geometric isomers of vitamin A with the then current theories of *cis–trans* isomerism, an α-ionone ring structure was discussed tentatively for isoretinenes-a and -b.[14] Bioassays of a sample of α-vitamin A aldehyde indicated little if any vitamin A activity. On the other hand, all the vitamin A isomers previously referred to exhibited substantial vitamin A activity. This evidence adds to the considerations presented by Robeson, *et al.*,[4] showing that the isoretinenes-a and -b are the 6-mono-*cis*- and the 2,6-di-*cis*-vitamin A aldehydes, respectively, and do not contain an α-ionone structure.

Acknowledgment is made to N. D. Embree and R. W. Lehman for advice, to C. D. Robeson for the isolation of the α-vitamin A alcohol, to H. A. Risley for the performance of vitamin A analyses of livers, to the Manufacturing Control Laboratory for chemical and spectrophotometric analyses, and to the Organic Chemistry Department for furnishing the crystalline vitamin A aldehydes.

ROCHESTER, N. Y.

(12) P. L. Harris, S. R. Ames and J. H. Brinkman, THIS JOURNAL, **73**, 1252 (1951).

(13) G. W. Wheland, "The Theory of Resonance and Its Application to Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1944, p. 134.

(14) R. Hubbard, R. I. Gregerman and G. Wald, *J. Gen. Physiol.*, **36**, 415 (1952–53).