UDC 547.538.1 : 577.161.12

146. Takuichi Miki and Yujiro Hara : Studies on Ethylenic Compounds. III.*1 Hydrazone-type Derivatives of Vitamin A Aldehyde.*2

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The studies on the action mechanism of vitamin A have indicated that retinene has a very close relationship to rhodopsin (visual purple). All-*trans* vitamin A alcohol is oxidized to aldehyde compound, i.e. retinal, in the presence of alcohol dehydrogenase *in vivo*, and is further converted to active retinal (neoretinal-b; 5,7-di-*cis* vitamin A aldehyde[1]) when light was absorbed in retina. It was found that rhodopsin was finally formed by combination with the amino group of opsin, the photoprotein.[2] Since hydrazines have the amino group, various hydrazine derivatives were reacted with retinal and crystalline derivatives were found to be produced easily. Physiological action of these derivatives would be of a great interest and the action was examined, together with other characteristics such as their stability.

Previously, Ball and others,[3] in order to prepare hydrazone of retinal, obtained retinal azine (I) in crystals of m.p. 135~145° by a reaction of hydrazine hydrate with retina. In this reaction, an organic acid was found to served as a good promotor and its application afforded a crystalline product of m.p. 189° in good yield. Though the melting point differed about 40° from that reported by Ball and others, the product obtained may be identical with retinal azine (I) from its elementary analysis and ultraviolet absorption spectrum (λ_{max} 465 mμ).*4 Acetic, maleic, and phthalic acids used in this reaction certified that an employment of organic acids increased a yield. (I) is very stable in the air forming reddish brown crystals like carotene. The toxicity was low and its physiological action was equivalent to that of 1 mole of vitamin A palmitate, although two retinylidene groups were contains in 1 mole of (I).

Nextly, retinal was reacted with various acylhydrazines prepared from aliphatic and aromatic acids. This reaction took place similarly to the synthetic vitamin A aldehyde and the melting point, and infrared and ultraviolet absorption spectra of the derivatives obtained agreed with those of the products derived from natural vitamin A. These properties are listed in Table I.

Apparently from this table, the ultraviolet absorption maxima of these acylhydrazone derivatives in ethanol are approximately at the same position to that of retinal when the substituent R was an aliphatic hydrocarbon residue, but the maxima were quite different when R is an aromatic residue. When R was a benzene ring, a shift of the maximum moved towards longer wave-length region in the presence of an electron-attracting group at the *para*-position but to the shorter wave-length region in the presence of an electron-repelling group at the *para*-positon. When R is a heteroaromatic ring, a shift of the maximum moved towards longer wave-length side. Such

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*1 This paper constitutes a part of a series entitled "Studies on Ethylenic Compunds" by Yasuo Abe.

*2 The process described in this work was patented. T. Miki, Y. Hara, Japan. pat. 2118 (1959), 11421 (1961).

*3 Juso-nishino-cho, Higashiyodogawa-ku, Osaka (三木卓一, 原 雄次郎).

*4 It has not been investigated yet whether this difference of melting point from that of the product obtained by Ball may be caused from its impurities. It may be understandable of forming *syn-anti* isomers.

1) J.M. Dietere, C.D. Robeson : Science, **120**, 219 (1954).
2) F.D. Collins, J.N. Green, R.A. Morton : Biochem. J., **56**, 493 (1954); L. Zechmeister : Experientia, **10**, 1 (1954).
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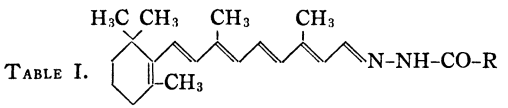


TABLE I.

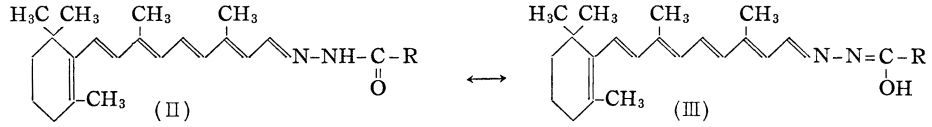
R	Acylradical in retinal acylhydrazone	Abbrev.	m.p. °C	Appearance[a]	U.V. λ_{max}^{EtOH} mμ	U.V. ε	Recrystn. solvent	Formula	Analyses (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Stability[b] (%)	LD_{50} (mg./kg.)	Biopotency[c]
CH_3	Acetyl		180	O Y needles	381	52,000	EtOH	$C_{22}H_{32}ON_2$	76.69	10.53	8.13	77.05	9.35		—	—	—
C_3H_7	Butyroyl	RBuH	178	Y scales	382	73,000	〃	$C_{24}H_{36}ON_2$	78.21	9.85	7.60	78.30	9.83	7.63	0	1700[d]	1/3
C_9H_{19}	Caprinoyl		126	Y needles	381	62,000	〃	$C_{30}H_{48}ON_2$	79.59	10.69		79.47	10.46		—	—	—
$C_{11}H_{28}$	Lauroyl		116	〃	381	63,000	〃	$C_{32}H_{52}ON_2$	79.94	10.90	5.83	79.87	10.60	5.90	—	—	—
$C_{13}H_{27}$	Myristoyl		106	〃	380	68,000	〃	$C_{34}H_{56}ON_2$	80.26	11.09	5.51	80.22	10.92	5.93	—	—	—
$C_{15}H_{31}$	Palmitoyl		96	Y scales	382	44,700	〃	$C_{36}H_{60}ON_2$							79	—	—
$C_{17}H_{35}$	Stearoyl		39～40	Y sandy	376	41,000	Me_2CO	$C_{38}H_{64}ON_2 \cdot H_2O$	78.29	11.41	4.81	78.43	11.23	4.86	—	—	—
$C_{17}H_{32}OH$	Ricinoloyl		95～98	Y	378	45,000	EtOH	$C_{38}H_{62}O_2N_2$	78.68	10.95		78.38	11.22		—	—	—
$C_5H_{11}O_5$	Gluconoyl	RGH	161	Y cubes	380	51,500	〃								—	—	<1/10
C_6H_5	Benzoyl	RBH	185	P Y needles	390	67,000	〃	$C_{27}H_{34}ON_2$	80.55	8.51	6.96	80.54	8.40	6.74	93.5	1000[e]	1/3～1/2
C_6H_4-OH(*p*)	*p*-Hydroxy-benzoyl		154	Y powder	380	47,700	—								54.6	—	active
C_6H_4-NH_2(*p*)	*p*-Amino-benzoyl	RABH	140	〃	385	56,000	—								71.5	4000	1
C_6H_4-NO_2(*p*)	*p*-Nitro-benzoyl	RNBH	174	O Y cubes	260 400	14,000 50,000	EtOH	$C_{27}H_{33}O_3N_3 \cdot C_2H_5OH$	70.56	7.96	8.51	70.15	7.60	8.73	100	—	<1/10
C_6H_4-COOH(*p*)	*p*-Carboxy benzoyl	RCBH	203	G B cubes	240 388	17,000 46,000	dil. EtOH	$C_{28}H_{34}O_3N_2 \cdot C_2H_5OH$	72.76	8.13	5.70	72.40	7.87	5.86	30.1	—	<1/10
C_5H_4N	Nicotinoyl		177	Y prisms	395	53,700	EtOH	$C_{26}H_{33}ON_3 \cdot C_2H_5OH$	74.79	8.74	9.35	75.02	8.77	9.14	—	—	active
C_5H_4N	Isonicotinoyl	RIBH	173	P Y prisms	396	48,900	〃								—	—	〃
$-CH_2\overset{+}{N}(CH_3)_3\ \overset{\vert}{Cl^-}$	Trimethyl ammonium chloride N-methyl-carbonyl		215 (dec.)	Y scales	382	55,000	〃								—	—	—
$-CH_2C_5H_5N \cdot Cl$	Pyridinium chloride N-methylcarbonyl		210 (dec.)	Y crystals	380	38,000	〃	$C_{27}H_{36}ON_3Cl$	71.42	7.99		71.87	7.86		—	—	

a) Color : Y=yellow, P=pale, G=grey, B=brown, O=orange

b) Stability is the residual rate after exposure of the sumple to the air at 30° in 90% relative humidity.

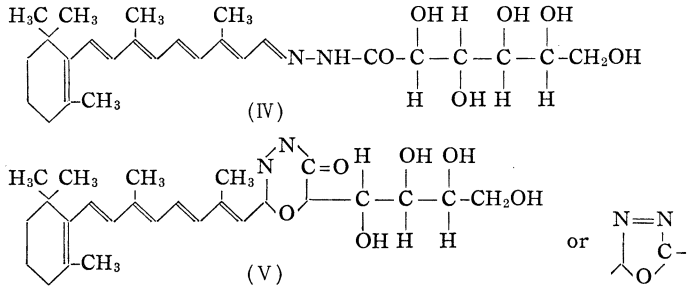
c) Biopotency against Vitamin A palmitate as 1. *d*) (1325～2145) *e*) (814～1230)

a phenomena were observed in the case of nicotinoyl- and isonicotinoylhydrazones and explained that the carbonyl group of the compound (II) is enolized to the form (III), an equilibrium is set up between (II) and (III), and this equilibrium seemed to move towards (III) when R was an aromatic residue.



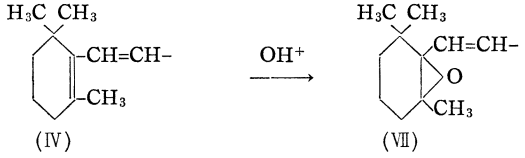
The compound designated as RCBH in Table I dissolved in sodium hydrogen carbonate solution to form a sodium salt which was unstable in aqueous solution. The compound RGH, having five hydroxyls in its molecule had been expected to show better solubility in water. The maximum of ultraviolet absorption in ethanol was observed at 380 mμ and at 322 mμ in aqueous solution, indicating a shift of ca. 60 mμ towards shorter wave-length region. It may be considered that this phenomenon is due to an addition of water to the double bond in -N=C- and thus intramolecular cyclization may be taken place in the structure of (V). When such a cyclization of the structure (V) is taken place, vitamin A activity will be lost, probably due to a failure of regenerating retinal. Solubility of (IV) in water is 6.6×10^{-4}(20°).

The product obtained by an application of the Girard-T reagent to retrodiene vitamin A aldehyde was a condensate of retinal with the Girard-T reagent. Majority



of these acylhydrazone derivatives show resistance to an air oxidation but, generally, compounds with R of aromatic residue seem to be more stable than those with aliphatic residue. The compounds RNBH, RBH, and RABH are especially stable.

At an initial stage of vitamin A oxidation, the double bond in the β-ionone ring is attacked by OH^+ and forms an epoxide (VI→VII). In the hydrazone derivatives of retinal,



an electron is attracted at the nitrogen atom by the inductive effect of the nitrogen atom adjacent to the carbon skeleton of vitamin A and electron density of the resonance system of carbon skeleton of vitamin A is lowered. This is considered to be responsible for preventing an attack of OH^+ observed at an early stage of vitamin A oxidation.

The compound RINH are expected to show, besides vitamin A activity, an antitubercular action similar to that of isonicotinic acid hydrazide (tested by the Institute of Microbiology, University of Osaka).

Experimental

Retinal Azine (I)——a) A mixture of 2.5 g. of retinal (purity, ca. 45%), prepared from natural vitamin A, 3 cc. of Ac_2O, and 1 cc. of hydrazine hydrate was stirred at room temperature for 3 hr., the crystals deposited were collected, and washed with EtOH, affording 1.6 g. of crude product. This was recrystallized from EtOH, containing a small amount of benzene to dark red needles, m.p. 189°. UV : $\lambda_{max}^{CHCl_3}$ 465 mμ (ε 99,900). *Anal.* Calcd. for $C_{40}H_{56}N_2$: C, 85.05; H, 9.99; N, 4.96; mol. wt., 564. Found : C, 85.26; H, 9.95; N, 5.34; mol. wt. (Barger), 650±50.

b) A mixed solution of 0.5 g. of phthalic anhydride, 0.5 g. of hydrazine hydrate, 5 cc. of EtOH, and 2 cc. of H_2O was added to 1.0 g. of retinal (purity, ca. 45%) dissolved in 5 cc. of EtOH, the mixture was heated on a water bath for 1 hr., and stirred at room temperature for 3 hr. The reaction mixture was diluted with 50 cc. of H_2O and 100 cc. of benzene, which was separated, and washed with NaCl solution. The organic layer was dried over anhyd. Na_2SO_4 and benzene was removed, leaving 0.5 g. of crude crystals. Recrystallization from EtOH containing a small amount of benzene afforded dark red needles, m.p. 189°, undepressed on admixture with an authentic sample of (I).

Retinal Acylhydrazone (II)——To a solution of 0.002 mole of retinal dissolved in 10 cc. of EtOH, a solution of 0.003 mole of acylhydrazine in 5~10 cc. of EtOH was added, the mixture was heated on a water bath for 3~5 hr., and concentrated under reduced pressure. A small amount of petr. ether (b.p. 80~100°) was added to the residue and the mixture was allowed to stand in a cold place. The crystals separated out were collected and recrystallized from a suitable solvent. These retinal acylhydrazones, in general, are comparatively easily soluble in polar solvents but suitable recrystallization solvent could not be found for the compounds ROBH and RABH (Table I). These compounds were, therefore, purified in the following manner.

The reaction mixture was concentrated under reduced pressure, about 50 volumes of Et_2O was added to the reddish brown oily residue, and the mixture was boiled on a water bath for a while. Et_2O solution was removed by decantation and about 50 volumes of H_2O was added to its residue. The mixture was stirred with warming on a water bath and aqueous solution was decanted. Repeated procedure for three times removed unreacted hydrazine and impurities in the starting material. The residue was dissolved in benzene and distilled to remove water, and the residue was dried under reduced pressure. This purification gave ROBH and RABH as a yellow powder.

Synthesis of RBuH (II : R=C_3H_7)——A mixture of 2.9 g. of synthetic vitamin A aldehyde prepared by the Takeda Process,[4] 1.5 g. of butyrylhydrazine, and 40 cc. of EtOH was boiled on a water bath for 3 hr., the solvent was distilled off, and the residue was boiled with 40 cc. of petr. ether (b.p. 60~80°) for 3 hr. This solution was concentrated and allowed to stand in a cold place, followed by the separation of 1.7 g. of crude crystals. Recrystallization from EtOH afforded pale yellow needles, m.p. 177°, undepressed on admixture with an authentic specimen. UV : λ_{max}^{EtOH} 381~382 mμ (ε 73,000).

RABH (Retinal *p*-Aminobenzoylhydrazone)——A solution of 16 g. of retinal (purity 45%), 9 g. of *p*-aminobenzoylhydrazine, and 400 cc. of EtOH was heated on a water bath for 3 hr. and the reaction mixture was concentrated under reduced pressure. The residue was diluted with 200 cc. of H_2O, stirred with warming, and water was decanted off. This procedure was repeated three times, 200 cc. of petr. ether (b.p. 60~80°) was added to the residue, and the mixture was boiled on a water bath. Petr. ether layer was decanted and this procedure was also repeated three times. The residue was dried under reduced pressure and 12 g. of brown powder was obtained. UV : λ_{max}^{EtOH} 385 mμ (ε 56,00).

Preparation of Parenteral Solution for the Test of Vitamin A Activity——A parenteral solution of NDGA was prepared by dissolving in sesame oil JP (sp. gr. 0.93) to make an 0.01% solution and filtered.

i) Vitamin A palmitate solution (80 U/cc.) : A solution of 182 mg. of vitamin A palmitate (1,320,000 U/g.=0.759 γ/U) (Riken Vitamin Company product) was dissolved in 280 g. of sesame oil JP.

ii) Vitamin A palmitate solution (20 and 40 U/cc.) : The above solution was diluted two-fold to make the 40 U/cc. solution and this was further diluted two-fold to make tho 20 U/cc. solution.

iii) RABH solution (50 U/cc.) : Two drops of EtOH was added to 3.79 g. of RABH (λ_{max}^{EtOH} 385 mμ (ε 56,000)) and this was dissolved in 2 cc. of Tween 80, and this mixture was dissolved in 140 g. of sesame oil. This can be heated to effect solution, if necessary.

The authors wish to thank Dr. S. Kuwata and Dr. T. Matsukawa for their helpful and stimulating interests in this work. Also our acknowledgements are made to Dr. T. Kobayashi and Dr. H. Mima for the performance of biopotency and stability test, respectively.

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Summary

It was found that the crystalline derivatives were readily obtainable when various hydrazine compounds were reacted with retinal. Many hydrazone-type derivatives of retinal were prepared and their physical properties, stability, toxicity and biopotency were examined.

All such derivatives are more stable than vitamin A palmitate, their toxicity being low, and some of them showed considerably higher vitamin A activity.

Retinal azine is obtained as crystals of m.p. 189° in a good yield when retinal is reacted with hydrazine-hydrate in the presence of an organic acid as a promotor. This compound is expected to have higher biological activity. *p*-Aminobenzoylhydrazone of retinal showed the biopotency equivalent to that of vitamin A.

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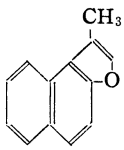
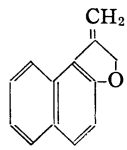
147. Issei Iwai and Junya Ide : Studies on Acetylenic Compounds. XXIII.*2 A New Ring Closure of 2-Propynyl Ethers.

(*Takamine Laboratory, Sankyo Co., Ltd.**1)

In the previous paper of this series, Okajima[1] reported the Claisen rearrangement of 3-phenoxy-1-propyne (I) and 3-(2′-naphthyloxy)-1-propyne (II). The Claisen rearrangement[2] of (I) had been investigated by Powell and Adams,[3] but no definite substance could be isolated either by refluxing diisopentyl ether (b.p. 170°) solution of (I) or by boiling (I) without solvent. Okajima reinvestigated the rearrangement of (I) in various conditions, but any definite substance was not obtained. On the other hand, by heating (II) at 208° for 20 minutes, he obtained only a small amount of pale yellow needles, m.p. 180°. Considering the naphthoic activity, he suggested one of the following structures for this compound from the results of elemental analysis and infrared spectrum :



(III)



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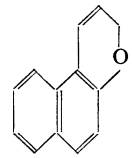

(V)

But a quantity of the sample was so small that he could not determine its structure.

In this paper, authors intend to clarify the chemical behavior of 3-(1′ or 2′-naphthyloxy)-1-propyne which are expected to give phenolic compounds by the Claisen rearrangement.

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