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Chemistry of Vitamin A. XXVII. The Decarboxylation of γ-Arylidene-β-methylglutaconic Acids[1]

BY JOHN D. CAWLEY AND DONALD R. NELAN

RECEIVED AUGUST 24, 1954

Heating γ-arylidene-β-methylglutaconic acids in a pyridine base containing cupric ion selectively eliminates the γ-carboxyl group and gives, in good yield, 5-aryl-3-methyl-2,4-pentadienoic acids. The reaction is stereospecific and gives exclusively the same geometric isomer that is obtained *via* the Reformatsky reaction between methyl γ-bromosenecioate and the corresponding aryl aldehyde. This isomer must have a 4-*cis* configuration. The *cis* bond is conjugated with the aryl group, an arrangement which usually causes large hypso- and hypochromic shifts, relative to the *trans* configuration, in the longest wave length band of the ultraviolet absorption spectra, but these *cis* compounds absorb with only moderately diminished intensity at slightly longer wave lengths than their *trans* isomers. The decarboxylation of the diacids obtained by condensing *trans*- and *cis*-β-ionylideneacetaldehyde with methyl β-methylglutaconate must take a different stereochemical course.

Three geometric isomers of 3-methyl-5-phenyl-2,4-pentadienoic acid (I, R = C_6H_5) are known. Kuhn and Hoffer[2] subjected benzylideneacetone to a Reformatsky reaction with methyl bromoacetate and obtained directly an unsaturated ester which, on saponification, yielded exclusively an acid Ia (R = C_6H_5) of m.p. 125°.[3] Iodine and light converted this to an isomer Ib (R = C_6H_5) of m.p. 160°. Kuhn and Hoffer showed that a variety of α,β-unsaturated ketones similarly yielded lower melting acids, isomerizable to and often accompanied by their higher melting forms. From considerations of melting point and light absorption data they tentatively assigned to the lower melting forms a 2-*cis*-4-*trans* configuration, and to the higher melting forms an all-*trans* configuration. The assignment of the 2-position to the *cis* bond is certainly reasonable since it is this bond which is newly created in the synthesis.

The third isomer Ic (R = C_6H_5), of m.p. 158°, has been obtained, together with the *trans* form Ib, by the Reformatsky reaction of benzaldehyde with methyl γ-bromosenecioate.[4] It, too, can be converted to the all-*trans* form Ib by iodine and light. It is at once apparent that the mere existence of a third geometric isomer of a dienoic acid I requires that it have a *cis* configuration at the 4-position; there is no alternative. Again, this seems reasonable, since it is this double bond which has been newly created. The configuration at the 2-position is uncertain. In the absence of any reports of or evidence for a fourth isomer, and of any specific evidence to the contrary, we shall consider it to be trans; *i.e.*, the compounds Ic are assumed to have a 2-*trans*-4-*cis* configuration.[5]

In exactly the same ways, three isomers of 3-

(1) Communication No. 204 from this Laboratory. Presented in part before the Division of Biological Chemistry of the 126th Meeting of the American Chemical Society, New York, New York, September, 1954.

(2) R. Kuhn and M. Hoffer, *Ber.*, **65**, 651 (1932).

(3) This isomer was also prepared by I. M. Heilbron, E. R. H. Jones, B. Julia and B. C. L. Weedon, *J. Chem. Soc.*, 1823 (1949), by another method. The failure of M. N. Shchukina and I. A. Rubtsov, *Zhur. Obshcheĭ Khim.*, **18**, 1645 (1948) to obtain this isomer by the same method may well be ascribed to their use of excess warm hydrochloric acid at a crucial point in the synthesis. Similarly, others who performed a Reformatsky reaction on benzalacetone but failed to obtain this isomer subjected the reaction mixture either to the prolonged action of hot mineral acid (E. P. Kohler and G. L. Heritage, *Am. Chem. J.*, **43**, 475 (1909); K. v. Auwers and J. Heyna, *J. prakt. Chem.*, **105**, 361 (1923) or to the action of iodine.[4b] Our work (see Experimental) substantiates completely that of Kuhn and Hoffer.

(4) (a) K. Ziegler, W. Schumann and E. Winkelmann, *Ann.*, **551**, 120 (1942); (b) R. Fuson and P. L. Southwick, THIS JOURNAL, **66**, 679 (1944); (c) S. H. Harper and J. F. Oughton, *Chemistry and Industry*, 574 (1950).

(5) The infrared spectra of the compounds I (R = C_6H_5) are consistent with the assigned configurations. The presumed all-*trans* isomer Ib shows a strong doublet at 10.4 and 10.45 μ, in the region characteristic of the symmetrically disubstituted *trans*-double bond. The presumed 2-*cis*-4-*trans* isomer Ia has a strong singlet at 10.4 μ, while the presumed 2-*trans*-4-*cis*- isomer Ic shows no absorption in this region.

TABLE I

5-ARYL-3-METHYL-2(*trans*),4(*cis*)-PENTADIENOIC ACIDS

Ic, R =	M.p., °C.	λmax,[a] mμ	εmax	λ'max, mμ	ε'max.	ε'/ε	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen,% Calcd.	Hydrogen,% Found
C_6H_5	158.5–159	308	27,700	230	14,000	0.506	88	..	..	..	..
p-ClC_6H_4	214–215	313	30,000	232.5	16,400	.547	93.5	64.7	64.8	5.0	4.8
p-$MeOC_6H_4$	151–152	331	28,300	238	13,900	.492	76	71.5	72.2	6.5	6.4
m-$NO_2C_6H_4$	213–213.5	300	30,000	222	18,400	.613	83	61.8	62.0	4.7	4.7
m-HOC_6H_4	176.5–177.5	308	23,500	~225–30	10,800	.460	83	70.6	69.6	5.9	6.2
p-$AcNHC_6H_4$	237–237.5	334	31,700	243	14,600	.461	85	68.6	68.6	6.2	6.1
p-$Me_2NC_6H_4$	205–206	379	26,100	261	12,900	.494	34.5	72.7	72.7	7.4	7.8
3,4-$CH_2O_2C_6H_3$	178–179	341	23,500	240	11,300	.482	81	67.2	67.6	5.2	5.4
		308	14,500								
3,4-$(MeO)_2C_6H_3$	159–160	339	24,600	243	12,400	.504	65	67.7	67.8	6.5	6.5
2,6-$Cl_2C_6H_3$	181–181.5	290	19,700	228	15,300	.777	97	56.1	56.5	3.9	3.9
C_6H_5CH=CH	192–192.5	336	45,800	245	10,800	.236	95	78.5	78.2	6.6	6.6

[a] All spectra were determined in absolute ethanol.

TABLE II

5-ARYL-3-METHYL-2(*trans*),4(*trans*)-PENTADIENOIC ACIDS

Ib, R =	M.p., °C.	λmax, mμ	εmax	λ'max, mμ	ε'max.	ε'/ε	εIc/εIb	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
C_6H_5	160.5–161	307	33,800	230	9,900	0.293	0.820	..	..	..	..
p-$MeOC_6H_5$[a]	178–179, 190	328	34,000	238	10,900	.321	.833	71.5	71.9	6.5	6.5
3,4-$CH_2O_2C_6H_3$	213	338	27,300	242	9,700	.355	.861	67.2	67.1	5.2	5.4
		307	17,300								
3,4-$(MeO)_2C_6H_3$	180.5–181.5	334	28,000	245	11,300	.403	.878	67.7	67.8	6.5	7.0
2,6-$Cl_2C_6H_3$	181–181.5[b]	288	24,400	229	16,450	.674	.808	56.0	56.0	3.9	4.0
C_6H_5CH=CH	199.5–200.5	333	52,300	242.5	6,500	.124	.876	..	..	..	..

[a] A. Lespagnol and J. Schmitt, *Bull. soc. chim. France*, 458 (1950), reported a m.p. of 181° for this compound. [b] The mixed m.p. with Ic (R = 2,6-$Cl_2C_6H_3$) was 154–169°.

methyl-7-phenyl-2,4,6-heptatrienoic acid (I, R = C_6H_5CH=CH) have been prepared. Cinnamylideneacetone and methyl bromoacetate yielded the 2-*cis*-4-*trans* isomer Ia, m.p. 169°, together with the all-*trans* form Ib, m.p. 203°,[2] while cinnamaldehyde and methyl γ-bromosenecioate gave the 2-*trans*-4-*cis* isomer Ic, m.p. 192°.[4a]

We have found that the γ-arylidene-β-methylglutaconic acids II (R = C_6H_5 and C_6H_5CH=CH), whose preparation from benzaldehyde and cinnamaldehyde and methyl β-methylglutaconate was described in the preceding paper,[6] yield, on elimination of the γ-carboxyl group, exclusively the 4-*cis* compounds Ic (R = C_6H_5 and C_6H_5CH=CH), of m.p. 158 and 192°, respectively. No other isomers are formed; the reaction is stereospecific, as is the condensation of the aldehyde with the glutaconate leading to II.[6]

All of the other previously described[6] γ-arylidene-β-methylglutaconic acids II similarly lose carbon dioxide to give the monoacids whose properties are listed in Table I. Four of these were isomerized by iodine and light to the all-*trans*[7] compounds (Table II), showing that in these cases the decarboxylation had also given *cis* compounds. It is presumed that *cis* compounds were formed in all cases, and that these all have the 4-*cis* configuration Ic, although the 2-*cis* isomers are all unknown and a direct comparison is therefore not possible. These presumptions are supported by the light absorption data which are discussed below.

(6) J. D. Cawley, THIS JOURNAL, **77**, 4125 (1955).

(7) The compound Ib (R = *p*-$MeOC_6H_4$) has a double melting point. This is evidence for its having an all-*trans* configuration, because only linear molecules show this phenomenon: *cf.* Y. Hirschberg, E. Bergmann and F. Bergmann, *ibid.*, **72**, 5120 (1950), and references there.

The decarboxylation is conducted simply by heating a solution of the glutaconic acids in a pyridine base containing cupric ion.[8] The reaction proceeds rapidly at 90–95°, and is completely selective toward the γ-carboxyl group. Table I shows that excellent yields of the monoacids Ic are obtained and, since the preparation of the glutaconic acids also proceeds in good yield,[6] these reactions constitute an excellent method for extending the chain of an aryl aldehyde by four carbon atoms.

The compound Ic (R = *p*-$Me_2NC_6H_4$) was obtained directly from the condensation of *p*-dimethylaminobenzaldehyde with methyl β-methylglutaconate; spontaneous decarboxylation of the intermediate diacid II (R = *p*-$Me_2NC_6H_4$) occurred. Similarly, the monoacid Ic (R = *p*-$NH_2C_6H_4$), λ_{max} 361 mμ and λ'_{max} 248 mμ, was obtained by the saponification of the diacid II (R = *p*-$AcNHC_6H_4$), but it was very unstable. A stable ethyl ester was smoothly formed by the simultaneous deacylation–esterification[9] of Ic (R = *p*-$AcNHC_6H_4$).

It was concluded[6] that the arylideneglutaconic acids have the 4-*cis* configuration shown in their formula, and their decarboxylation to the 4-*cis* monoacids Ic therefore proceeds with retention of this configuration. This parallels the decarboxylation of *cis*-stilbene-α-carboxylic acids, which yield,

(8) The decarboxylation was originally carried out, with success, with pyridine and copper powder, or by heating at 145–150° with quinoline alone. The preferred conditions using 2,4-lutidine and cupric acetate, described in the Experimental section, are the result, principally, of work by Dr. Max Stern and Mr. George Fletcher.

(9) J. P. van Roon, P. E. Verkade and B. M. Wepster, *Rec. trav. chim.*, **70**, 1105 (1951).

under conditions somewhat similar (quinoline and copper chromite) but far more brutal (230°) than those employed here for the decarboxylation of II, exclusively *cis*-stilbenes.[10] Although the arylideneglutaconic acids are (substituted) vinylogs of arylidenemalonic acids, the decarboxylation of the former cannot proceed by the addition–elimination mechanism which Corey and Fraenkel[11] have recently shown is valid for the latter; such a mechanism should and does lead to monoacid products in which geometric isomers are present in equilibrium proportions.

Ultraviolet Spectra.—In Fig. 1 are shown the ultraviolet absorption spectra of the three isomers Ia,[12] Ib, and Ic (R = C_6H_5). The extinction coefficients for the long wave length band, ϵ_{max}, are in the ratio Ib:Ia:Ic as 100:90:82. The λ_{max} of Ia (306 mμ) lies slightly below that of Ib (307 mμ), which, in turn, is below the λ_{max} of Ic (308 mμ). These differences, although small, are believed to be significant.[13]

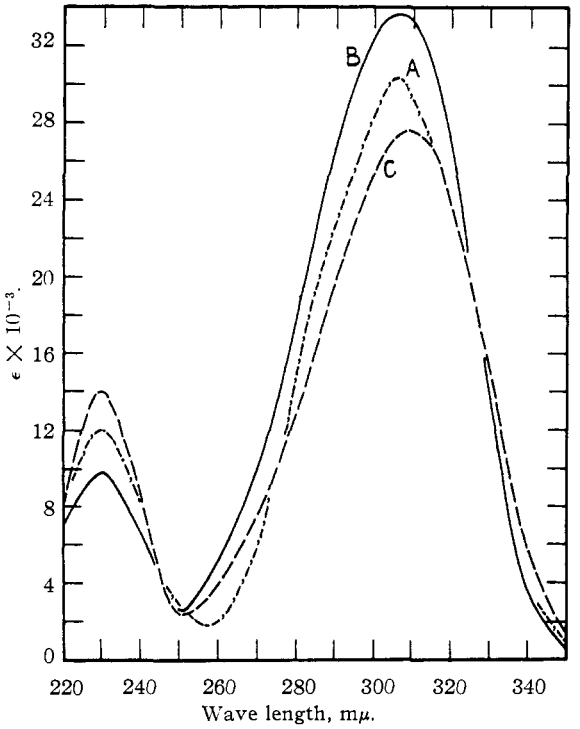


Fig. 1.—Absorption spectra in ethanol of the isomers of 3-methyl-5-phenyl-2,4-pentadienoic acid (I, R = C_6H_5): curve A, 2-*cis*-4-*trans* isomer Ia; curve B, 2-*trans*-4-*trans* isomer Ib; curve C, 2-*trans*-4-*cis* isomer Ic.

The λ_{max} of the other all-*trans* Ib compounds in Table II also lie 2–5 mμ below the λ_{max} of the corresponding compounds Ic in Table I, in accordance with the assumption that the latter have a 4-*cis* configuration. The ratios of extinction coefficients, $\epsilon_{Ic}/\epsilon_{Ib}$, given in Table II, also support this conclusion; on the average, *trans*:*cis* as 100:84.6.[14]

Except for the relatively small variations just discussed, both λ_{max} and ϵ_{max} of all of the compounds I, including the 4-*cis* isomers Ic, are normal for compounds having this conjugated system; *trans,trans*-5-phenyl-2,4-pentadienoic acid has λ_{max} 307 mμ, ϵ_{max} 36,700,[6] and piperic acid (*trans,trans*-5 - (3,4 - methylenedioxyphenyl) - 2,4 - pentadienoic acid) has λ_{max} 340 mμ, ϵ_{max} 29,000.[15] But Zechmeister[16] has pointed out that the assumption of a *cis* configuration by an unsubstituted double bond conjugated with a benzene ring gives rise to such hindrance between an ortho- and a chain-hydrogen atom that "an approximately planar configuration becomes impossible; the deviation will be about 52.5°." This loss of coplanarity results, normally, in large hypsochromic and hypochromic displacements of the long wave length band characteristic of the conjugated system. Thus, *trans*- and *cis*-propenylbenzene have λ_{max} 250 and 240.6 mμ, and the extinction coefficients are in the ratio *trans*:*cis* as 100:79.8.[17] Similarly, *trans*-1-phenylbutadiene has λ_{max} 280 mμ, while the *cis* isomer has λ_{max} 268 mμ, and the extinction ratio is 100:61.7.[18] Again, *trans,trans*-, *trans,cis*- and *cis,cis*-1,4-diphenylbutadiene have λ_{max} 328, 313 and 296 mμ, and the extinction ratios are 100:54:53.[19]

These profound effects are the result of hindrance between ortho- and chain-hydrogen atoms. But the 4-*cis* form of a compound I (*i.e.*, Ic) involves hindrance between an ortho-hydrogen atom and the 3-methyl group on the chain,[20] so that in these compounds a still larger effect would be expected. This expectation was fulfilled for the precursors II of Ic. The deviation from coplanarity is virtually 90° about the 3–4 single bond, so that the compounds II absorb maximally at far shorter wave lengths than would be expected for compounds having this conjugated system.[6] It is therefore surprising that upon the decarboxylation of II, an operation which does not affect the basic geometry of the molecule in any easily seen manner, an apparently (judging by the light absorption properties) nearly coplanar compound Ic results. These compounds are apparently an exception to Zechmeister's generalization quoted above.

Figure 1 and Tables I and II show that all of the compounds Ia, Ib and Ic have a second absorption maximum, λ'_{max}, at lower wave lengths than the main band. The intensity, ϵ'_{max}, of this second band increases in the order Ib < Ia < Ic, while its position remains the same. Braude, *et al.*,[21] have

(10) T. W. J. Taylor and P. M. Hobson, *J. Chem. Soc.*, 181 (1936); P. Ruggli and A. Staub, *Helv. Chim. Acta*, **20**, 37 (1937); G. B. Bachman and R. I. Hoaglin, *J. Org. Chem.*, **6**, 134 (1941).

(11) E. J. Corey and G. Fraenkel, THIS JOURNAL, **75**, 1168 (1953).

(12) Compound Ia (R = C_6H_5), prepared according to Kuhn and Hoffer,[2] was found by us to have λ_{max} 306 mμ, ϵ_{max} 30,400 and λ'_{max} 230 mμ, ϵ'_{max}, 12,100.

(13) The spectra were taken on a Model 11M Cary Recording Spectrophotometer, carefully calibrated, and operated by skilled personnel.

(14) This appears to rule out the possibility that the Ic molecules have assumed the sterically less-hindered 3-s-*cis* configuration (*cf.* Fig. 1B of the preceding paper); ϵ_{max} of s-*cis* compounds is only *ca.* 50% of the ϵ_{max} of their *s-trans* isomers (D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.*, 257 (1951).

(15) F. S. Spring and J. Stark, *ibid.*, 1177 (1950).

(16) L. Zechmeister, *Chem. Revs.*, **34**, 267 (1944).

(17) R. Y. Mixer, *et al.*, THIS JOURNAL, **75**, 4094 (1953).

(18) O. Grummitt and F. J. Cristoph, *ibid.*, **73**, 3479 (1951).

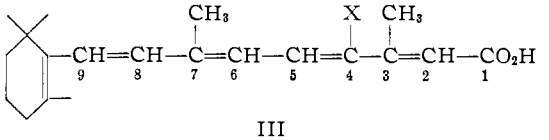
(19) J. H. Pinckard, B. Wille and L. Zechmeister, *ibid.*, **70**, 1938 (1948).

(20) The situation is diagrammed in Fig. 1A of the preceding paper.

(21) E. A. Braude, E. R. H. Jones, *et al.*, *J. Chem. Soc.*, 1890 (1949); E. A. Braude, T. Brunn, *et al.*, *ibid.*, 1419 (1952).

pointed out that moderate hindrance to coplanarity in an extended conjugated system may leave the λ_{max} characteristic of the entire system unaffected, but may reduce the intensity of absorption at this wave length, while simultaneously absorption at lower wave lengths, arising from "partials," appears. The expected partial for the compound Ic is that of the corresponding styrene, just as the partial of II is the corresponding cinnamic acid enclosed in dotted lines in its formula. For II, the observed absorption spectra are due entirely to this partial,[6] for the reasons just discussed, but this is not true for the compounds Ic; styrene has λ_{max} 244 mμ,[22] and its derivatives have the following maxima: *p*-MeO, 262 mμ; *p*-Cl, 258 mμ; 2,6-Cl_2, 244 mμ.[23] The λ′ bands of the corresponding derivatives of I lie well below these values, so that not only are these λ′ bands not partials arising from steric hindrance, but also no such partial is present, as might have been anticipated.

trans- and *cis*-β-ionylideneacetaldehyde condense with methyl β-methylglutaconate to give diacids III (X = CO_2H), designated as C-diacid and D-



III

diacid.[24] These have the configuration 2-*trans*(?)-4-*cis*-6-*trans* and 2-*trans*(?)-4-*cis*-6-*cis*.[6] Decarboxylation of these C- and D-diacids produces C- and D-vitamin A acids[24] (III, X = H). If these decarboxylations also proceeded with retention of the configurations of the diacids, the C- and D-vitamin A acids would contain a *cis* bond at the "forbidden"[25] 4-position. Since the actual degree of spatial interference in these would be less than that in the 4-*cis* aryl acids Ic (*cf.* Figs. 1A and D of the preceding paper) this condition, *per se*, would be no bar to their existence. Further, the C- and D-vitamin A acids are isomerized by iodine to the all-*trans*- and 6-*cis*-vitamin A acids,[24] and the spectral relationships of the C- to the all-*trans* and of the D- to the 6-*cis*-acids are quite similar to the relationships of the aryl acids Ic to their *trans* isomers Ib. But the presumed 4-*cis* or "forbidden" C- and D-isomers of vitamin A compounds can be obtained by chemical means from the all-*trans* form. This is true for the vitamin A acids,[24] for the alcohol,[26] and especially for the aldehydes.[27] Since Pauling[28] has pointed out that what is actually forbidden is the attainment of, *e.g.*, the 4-*cis* form of a compound III from its *trans* isomer by chemical or thermal means, and since this theoretical conclusion is supported by experiment,[29] it becomes impossible to consider a 4-*cis* structure for the C- and D-isomers of vitamin A compounds. Indeed, the interconversion of the stereoisomers of vitamin A compounds by chemical means is the foundation for the detailed configurational assignments in ref. 24, for once isomerism about only the allowed positions 2 and 6 need be considered,[30] the assignments follow unambiguously from the properties, inter-relationships, and methods of synthesis of the four isomers. The difference in stereochemical behavior of the diacids III (X = CO_2H) upon decarboxylation as compared to the aryl diacids II is probably to be sought in the mechanism of the reaction. It may be significant that the decarboxylation of III (X = CO_2H) requires more drastic conditions (2 hr. at 120–125°[24]) than does the decarboxylation of II.

Acknowledgments.—Absorption spectra were determined under the direction of Mr. Albert Besançon by members of the Manufacturing Control Laboratory of this Company. Analyses were performed by the Microanalytical Laboratory of the Eastman Kodak Company under the direction of Mr. Donald Ketchum.

Experimental[31]

Preparation of Ia (R = C_6H_5).—To 36.0 g. (0.55 atom) of 80-mesh granulated zinc, 50 ml. of dry benzene and a crystal of iodine there was added with stirring so as to maintain a smooth reflux a solution of 92.0 g. (61.0 ml., 0.55 mole) of ethyl bromoacetate and 73 g. (0.50 mole) of benzalacetone in 250 ml. of dry benzene. When the addition was complete the mixture was refluxed 15 min., cooled, and 200 ml. of 20% acetic acid added. Ether was added and the organic layer separated and washed with dilute acetic acid, water, sodium bicarbonate and water. After drying, evaporation of the solvents gave 114 g. of oil. This was dissolved in 500 ml. of dry benzene and refluxed in a Dean–Stark apparatus with 2.38 g. of *p*-toluenesulfonic acid monohydrate for 40 min., when 7.2 ml. (0.40 mole, 80%) of water had collected. The material recovered from the benzene was distilled in nitrogen, giving 61.6 g. (57%) of the ester of Ia (R = C_6H_5) b.p. 160–163° (4 mm.). This (0.285 mole) was dissolved in 280 ml. of ethanol, a solution of 32 g. (0.57 mole) of potassium hydroxide in 40 ml. of water was added, and after standing overnight at room temperature the mixture was heated nearly to boiling and let stand 3/4 hr. After dilution with 1600 ml. of water and extraction with ether, acidification gave 47.7 g. of product. One crystallization from 200 ml. of benzene and 150 ml. of Skellysolve B gave 41.6 g. of Ia (R = C_6H_5) of m.p. 125–125.5°. From the mother liquor only material of the same melting point was isolated.

Decarboxylation of γ-Arylidene-β-methylglutaconic Acids (II) to 5-Aryl-3-methyl-2(*trans*),4(*cis*)-pentadienoic Acids (Ic).—The arylideneglutaconic acid[6] and 5 volumes of a solution of 5 g. of cupric acetate per l. of 2,4-lutidine were heated under reflux on the steam-bath with swirling until the solid had dissolved. Carbon dioxide was evolved vigorously from the hot solution for 15–20 min., on a molar scale, except with II (R = 2,6-$Cl_2C_6H_3$), where the completion of the reaction required 1.5 hr. Heating was continued for 1/2 hr. after the evolution had subsided. Most of the lutidine was distilled off in an aspirator vacuum in nitrogen, and the residue was diluted with water and acidified to congo red with concd. hydrochloric acid. The solid product Ic was filtered, washed thoroughly with water, dried and crystallized from ethanol or ethanol–water.

Preparation of Ic (R = *p*-$Me_2NC_6H_4$).—To 52 g. (0.35 mole) of *p*-dimethylaminobenzaldehyde and 85 g. of 85%

(22) Y. Hirshberg, THIS JOURNAL, **71**, 3241 (1949).

(23) H. A. Laitinen, F. A. Miller and T. D. Parks, *ibid.*, **69**, 2707 (1947).

(24) C. D. Robeson, J. D. Cawley, L. Weisler, M. H. Stern, C. C. Eddinger and A. J. Chechak, *ibid.*, **77**, 4111 (1955).

(25) L. Pauling, *Fortschr. Chem. org. Naturstoffe*, **3**, 203 (1939).

(26) C. D. Robeson and J. G. Baxter, THIS JOURNAL, **69**, 136 (1947).

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

(28) L. Pauling, *Helv. Chim. Acta*, **32**, 2241 (1949).

(29) L. Zechmeister and F. J. Petracek, THIS JOURNAL, **74**, 282 (1952).

(30) Isomerism at position 8 is improbable; the *trans* form is already quite hindered (*cf.* W. Oroshnik, G. Karmas and A. D. Mebane, THIS JOURNAL, **74**, 295 (1952), and references cited there).

(31) Melting points where taken on a 3-in. immersion thermometer in the apparatus described by May, *Anal. Chem.*, **21**, 1427 (1949), and may be considered to be corrected

pure methyl β-methylglutaconate[6] (0.42 mole) in 20 ml. of methanol, there was added a solution of 111 g. of 85% potassium hydroxide (1.68 moles) in 450 ml. of methanol. After 24 hr. at room temperature the mixture was cooled to 5° and the potassium salt of II (R = *p*-$Me_2NC_6H_4$) filtered; the yield was 60.3 g. (49.0%). This was dissolved in 240 ml. of water and approximately two equivalents of hydrochloric acid were added. The resulting solid (41.7 g.) was filtered and the filtrate made nearly acid to congo red, precipitating an additional 21.2 g. of product. The combined solids were crystallized thrice from 1:1 ethanol–acetone to give 27.8 g. of red-orange laths, m.p. 200–201°. The analytical sample was again crystallized from dioxane. The filtrates contained only a red-brown gum.

Preparation of Ethyl 5-(*p*-Aminophenyl)-3-methyl-2,4-pentadienoate.—Dry hydrogen chloride was added to a suspension of 4.9 g. (0.02 mole) of Ic (R = *p*-$AcNHC_6H_4$) in 160 ml. of ethanol until 3.7 g. (0.1 mole) had been absorbed. The mixture was then refluxed for 3 hr., complete solution occurring at once. Most of the ethanol was removed by evaporation under nitrogen, water was added to the residue, and, after ether extraction, the aqueous layer was made alkaline with solid potassium hydroxide. The oil which separated was extracted with ether, whose evaporation left 3.8 g. (82.5%) of crude product. This was chromatographed from benzene on a column of Alorco grade F-20 alumina. The main golden yellow zone was separated and eluted with ether to give 3.3 g. (72%) of the product as a yellow viscous oil. It was characterized as the picrate, prepared in ethanol in 82% crude yield, which formed yellow leaves, m.p. 142.5–143.5° after recrystallization from methanol.

Anal. Calcd. for $C_{20}H_{20}N_4O_9$: N, 12.2. Found: N, 12.3.

It is possible that the hydrogen chloride caused stereoisomerization, so that no configuration is assigned to this compound.

Rearrangements of the 4-*cis* Acids Ic to their *trans* Isomers Ib.—The several 4-*cis* acids examined were found to vary in their stability to iodine and light, so that in no case can the described procedure be considered the optimum.

The compounds Ic (R = *p*-$MeOC_6H_4$, 3,4-$CH_2O_2C_6H_3$, 3,4-$(MeO)_2C_6H_3$ and 2,6-$Cl_2C_6H_3$) were treated thus: the compound was dissolved in 2 volumes of methanol and 5 volumes of benzene (10 volumes for R = 3,4-$CH_2O_2C_6H_3$) containing 5% of the weight of the compound of dissolved iodine, and the solution was irradiated for 30 min. from below with a mercury vapor lamp at a distance of 3 cm., the heat from the lamp being sufficient to maintain a gentle reflux. The product which separated at 5° was crystallized from ethanol. The yields varied from 84% for R = 3,4-$CH_2O_2C_6H_3$ to 25% for R = 3,4-$(MeO)_2C_6H_3$.

The compounds Ic (R = C_6H_5 and $C_6H_5CH{=}CH$) were destroyed under the above conditions. The former was dissolved in 20 volumes of ether and 20 volumes of benzene containing 1% of the weight of the compound of dissolved iodine. After 4 hr. at room temperature in diffuse daylight an additional 1% of iodine in a small amount of benzene was added, and after 2 hr. more the solution was washed with thiosulfate and water, the ether removed by distillation, and ligroin added to the distilland to crystallization at the b.p. Two recrystallizations of the resulting solid from benzene gave 50% of Ib (R = C_6H_5), m.p. 157–158°; the mixed m.p. with Ic (R = C_6H_5) was 123–146°. The m.p. can be raised to 160.5–161° by further crystallizations from benzene and ethanol.

Ic (R = $C_6H_5CH{=}CH$) was treated with 1% of iodine in 75 volumes of ether and 37.5 volumes of benzene for 2 hr. at room temperature in diffuse daylight, then 1% more of iodine was added and, after a further 1.5 hr., the mixture was worked up by washing with thiosulfate and water and removing most of the ether by distillation. There crystallized from the distilland 80% of Ib (R = $C_6H_5CH{=}CH$), m.p. 196–198°, raised to 199.5–200.5 by recrystallization from ethanol. The compound crystallizes beautifully in pale yellow, large, very thin, rhombic plates, exactly as described by Kuhn and Hoffer.[2]

ROCHESTER, NEW YORK

[COMMUNICATION NO. 209 FROM THE RESEARCH LABORATORIES, DISTILLATION PRODUCTS INDUSTRIES, DIVISION OF EASTMAN KODAK COMPANY]

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

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

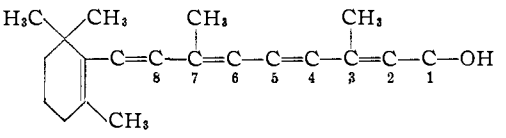
RECEIVED AUGUST 24, 1954

The physiological potencies of three new isomers, 6-mono-*cis*, 2,6-di-*cis* and 2,4-di-*cis*, of vitamin A acetate as well as of neo (2-mono-*cis*) vitamin A acetate have been determined using standard vitamin A bioassay procedures. Neovitamin A acetate (2-mono-*cis*) has a biopotency of 2,190,000 units/gram. The 6-mono-*cis*-vitamin A acetate has a biopotency of 634,000 units/gram; the 2,6-di-*cis* isomeric acetate has a biopotency of 688,000 units/gram; and the 2,4-di-*cis* isomeric acetate has a biopotency of 679,000 units/gram. The 6-mono-*cis*, 2,6-di-*cis* and 2,4-di-*cis* isomeric vitamin A acetates are about 23% as active as all-*trans*-vitamin A acetate.

Five isomers of vitamin A (all-*trans*, neo (2-mono-*cis*), 6-mono-*cis*, 2,6-di-*cis* and 2,4-di-*cis*) have now been characterized chemically and physically.[2] All-*trans*-vitamin A acetate has been isolated in crystalline form.[3] Neovitamin A acetate was first described by Baxter and Robeson[4] and was prepared from crystalline neovitamin A alcohol. The comparative biopotencies of all-*trans* and neovitamin A acetates have been previously reported by Harris, Ames and Brinkman.[5] Robeson, *et al.*,[6] have recently synthesized the 6-mono-*cis*- and 2,6-di-*cis*-vitamin A acetates. The aldehyde corresponding to the 2,4-di-*cis*-vitamin A was first described by Hubbard and Wald[7] and more recently by Dieterle and Robeson.[8] The present report summarizes the results of rat bioassays of the five isomeric vitamin A acetates.

(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) The steric configurations of the isomers can be identified according to the following numbering system



(3) J. G. Baxter and C. D. Robeson, THIS JOURNAL, **64**, 2407 (1942).

(4) J. G. Baxter and C. D. Robeson, *ibid.*, **69**, 136 (1947).

(5) P. L. Harris, S. R. Ames and J. H. Brinkman, *ibid.*, **73**, 1252 (1951).

(6) C. D. Robeson, J. D. Cawley, L. Weisler, M. H. Stern, C. C. Eddinger and A. J. Chechak, *ibid.*, **77**, 4111 (1955).

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

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