posed by this procedure consistently gave camphor–fenchone ratios in the range of 40–60:60–40%. If, however, the ozonide was decomposed in the alternate manner mentioned above, the ratio of camphor to fenchone was changed to the range, 15–20:85–80%. As a check one of the ozonization mixtures was divided into two portions and each was decomposed with and without steam distillation. The consistency of the result is shown in Table I.

The basic aqueous solution was acidified with hydrochloric acid and extracted with ether. Removal of ether from the dried solution left the mixed acids as an oil.

DAYTON 7, OHIO

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[CONTRIBUTION NO. 2075 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

# The Hydrolytic Cleavage Products of Boron Trifluoride Complexes of β-Carotene, Some Dehydrogenated Carotenes and Anhydrovitamin $A_1$

BY F. J. PETRACEK AND L. ZECHMEISTER

RECEIVED JANUARY 9, 1956

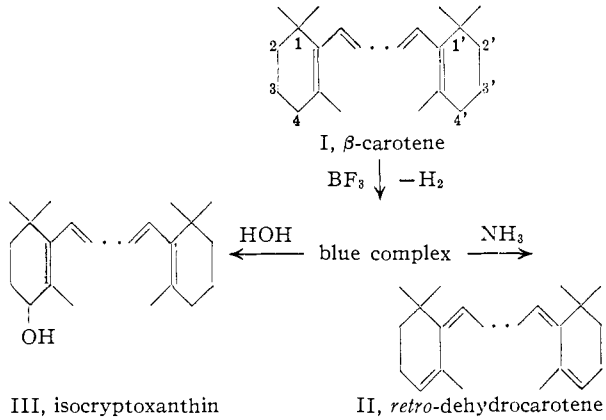
β-Carotene in chloroform when treated with $BF_3$-etherate yielded, upon hydrolysis of the blue complex, isocryptoxanthin, whereby the trifluoride had acted first as a dehydrogenating agent. From the 3,4-dehydro-α-carotene and 3,4-dehydro-β-carotene complexes the corresponding 4-hydroxy derivatives were obtained. *retro*-Bisdehydrocarotene yielded 2-hydroxy-3,4-dehydro-β-carotene. By dehydrating the latter compound with HCl–chloroform, the *retro*-bisdehydrocarotene was recovered. The hydrolysis of the blue complex of anhydrovitamin $A_1$ did not result in the recovery of the vitamin but in the formation of a new isomer, very probably 4-hydroxy-axerophthene which, under the influence of acid chloroform, dehydrated to anhydrovitamin $A_1$.

As reported earlier[1] a treatment of β-carotene (I), $C_{40}H_{56}$, with $BF_3$-etherate, followed by hydrolysis, resulted in the shortening of the chromophore and the formation of two new crystalline pigments. A similar treatment of a dehydrogenation product of β-carotene, *viz.*, *retro*-dehydrocarotene (II), $C_{40}H_{54}$,[2] yielded isocryptoxanthin (4-hydroxy-β-carotene), a structural isomer of naturally occurring cryptoxanthin.[3] Both conversions were carried out in hexane solution.

It was observed recently[4,5] that when commercial chloroform (containing 1% alcohol) was used, instead of carbon tetrachloride, as a solvent in some dehydrogenation processes, the course of the reaction was altered. The same medium, which allowed the use of higher concentrations in a one-phase system, has now been applied in the $BF_3$ experiments described below.

In the case of *retro*-dehydrocarotene (II) the effect of the change from hexane to chloroform was negligible, although the rate of the complex formation increased very considerably. However, the hydrolysis of the β-carotene–$BF_3$ complex, formed in chloroform, resulted in the isolation of isocryptoxanthin (III) instead of the two pigments mentioned.[1] This conversion so far as we know represents the first direct introduction of a hydroxyl group into the β-carotene molecule, in contrast to the indirect route, requiring dehydrogenation to *retro*-dehydrocarotene as a first step.[3]

We propose that, under the conditions applied, boron trifluoride acts on β-carotene first as a dehydrogenating agent forming a complex not with the starting material but with a dehydrogenated carotene. Indeed, when the blue complex was cleaved with dry ammonia (instead of water) *retro*-dehydrocarotene (II) was obtained (Scheme 1).




SCHEME 1

The dehydrogenating action of boron trifluoride would parallel a similar effect caused by another "Lewis acid," *viz.*, antimony trichloride, whose blue β-carotene complex yields on hydrolysis *retro*-dehydrocarotene.[6]

In connection with these experiments a reinvestigation has been carried out of the respective boron trifluoride complexes of 3,4-dehydro-α-carotene, $C_{40}H_{54}$, and 3,4-dehydro-β-carotene, $C_{40}H_{54}$. These pigments had been obtained by Karmakar and one of the writers[7] when reacting carotenes with N-bromosuccinimide in carbon tetrachloride solution yields of crystalline substance, 3.8 and 1.4%. By applying chloroform as a solvent and dehydrobrominating with N-phenylmorpholine, these yields have now been increased to 14.5 and 2%.

(1) L. Wallcave, J. Leemann and L. Zechmeister, *Proc. Natl. Acad. Sci.*, **39**, 604 (1953).

(2) This compound was termed earlier "dehydro-β-carotene"; *cf.* R. Kuhn and E. Lederer, *Ber.*, **65**, 637 (1932); P. Karrer and G. Schwab, *Helv. Chim. Acta*, **23**, 578 (1940); H. H. Inhoffen and G. Raspé, *Ann.*, **594**, 165 (1955). The name "*retro*-dehydrocarotene" was proposed by the latter authors.

(3) L. Wallcave and L. Zechmeister, THIS JOURNAL, **75**, 4493 (1953).

(4) F. J. Petracek and L. Zechmeister, *ibid.*, **78**, 1427 (1956).

(5) F. J. Petracek, Thesis, California Institute of Technology, 1956.

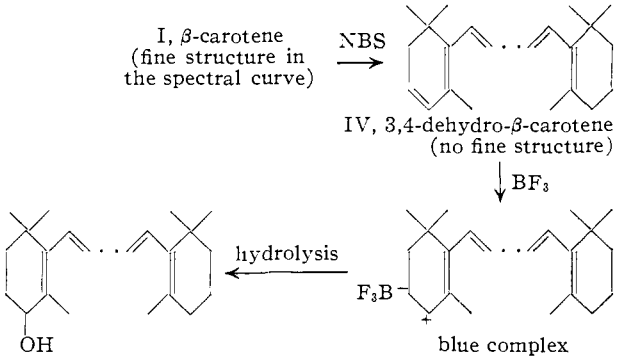
(6) A. E. Gillam, I. M. Heilbron, R. A. Morton and J. C. Drummond, *Biochem. J.*, **26**, 1174 (1932); P. Karrer and G. Schwab, ref. 2; *cf.* P. Meunier and A. Vinet, "Chromatographie et Mésomérie," Paris, Masson & Cie., 1947, p. 79.

(7) G. Karmakar and L. Zechmeister, THIS JOURNAL, **77**, 55 (1955).

As shown earlier,[7] when the boron trifluoride complex of either of these two dehydro compounds was hydrolyzed, the fine structure of the spectral curve (visible region), lost during the dehydrogenation process, reappeared. This then unexpected phenomenon was tentatively attributed to a double bond migration, and the resulting compounds were (incorrectly) defined as hydrocarbons. Larger-scale experiments have now shown that the cleavage of the 3,4-dehydro-α-carotene and 3,4-dehydro-β-carotene complexes yielded, respectively, 4-hydroxy-α-carotene and 4-hydroxy-β-carotene, each possessing the spectrum of the corresponding carotene. Hence, the whole reaction sequence includes the formation of a 3,4-double bond and its subsequent hydration, according to Scheme 2.

An analogous scheme should be valid for the conversion of α-carotene.

These observations are in accordance with the conversion of 4-keto-3′,4′-dehydro-β-carotene to its 4′-hydroxy derivative, *via* the $BF_3$-complex, observed in our laboratory.[4,5]



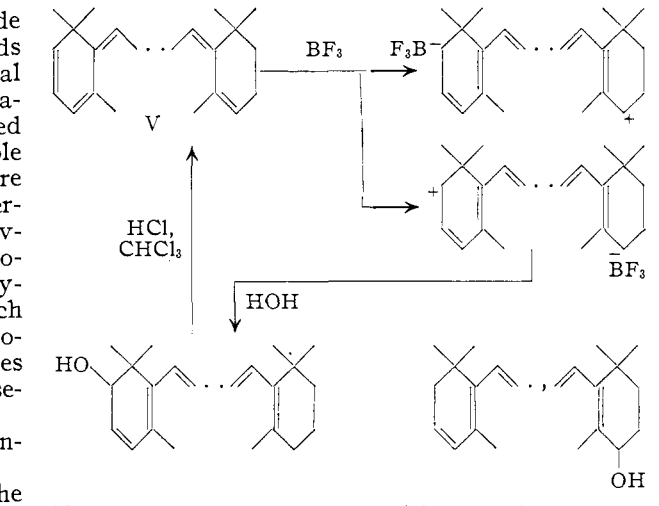

SCHEME 2

Another, highly colored dehydrogenation product of β-carotene, termed *retro*-bisdehydrocarotene, $C_{40}H_{52}$, was first prepared in collaboration with Wallcave,[3] and later shown[8] to have the non-symmetrical structure (V). Since in this instance the molecule contains two different end groups, *a priori* two boron trifluoride complexes could be formulated (Scheme 3), one yielding on hydrolysis the unknown 2-hydroxy-3,4-dehydro-β-carotene (VI) and the other the already known 4′-hydroxy isomer (VII).[4]

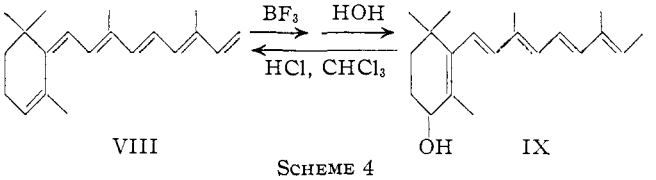
The experiment showed that the main product of the hydrolysis (yield, 30%) could be easily distinguished from the 4′-hydroxy compound by its crystal form, melting point, decreased adsorption affinity and slightly diminished hypophasic character. The two latter features are explained by the spatially screened position of the 2-hydroxy group. As expected, this allylic group was easily converted into an allylic ether in acidic methanol.[4] Furthermore, a dehydration with HCl–chloroform resulted in the recovery of *retro*-bisdehydrocarotene (V); yield 70% (Scheme 3).

That the conversions just described are not restricted to $C_{40}$-carotenoids, was shown, in small-scale experiments, by the behavior of anhydrovitamin $A_1$, $C_{20}H_{28}$ (VIII).[9] Its blue $BF_3$ complex, when hydrolyzed, did not revert to vitamin $A_1$, as might have been expected, but yielded an isomer, very probably 4-hydroxyaxerophthene (IX). The ultraviolet spectrum of this chromatographically homogeneous (but not yet crystallized) product coincided (qualitatively) with that of the vitamin. However, the two infrared curves were markedly different, especially in the C–O stretching region (9–10 μ). The 4-hydroxy isomer had a peak of medium intensity at 9.75 μ that was absent from the vitamin $A_1$ curve. Both compounds showed a band at 2.75 μ (O–H stretching).

When partitioned between hexane and 95% methanol, the new polyene-alcohol was somewhat less hypophasic than vitamin $A_1$. Upon a treatment with HCl–chloroform, it was dehydrated to anhydrovitamin $A_1$ (VIII) (Scheme 4).




SCHEME 3




SCHEME 4

## Experimental

The materials used (including adsorbents) and the methods employed were specified in our recent paper.[4] When no adsorbent is mentioned, the chromatographic column was composed of lime–Celite 2:1. In the visible and ultraviolet regions all spectral data refer to hexane solutions, and infrared readings to carbon tetrachloride (1% solutions, 1-mm. cells, Perkin–Elmer double beam instrument, model 21).

**Isocryptoxanthin (4-Hydroxy-β-carotene).** (a) **From β-Carotene.**—To a solution of 100 mg. of β-carotene in 100 ml. of chloroform (Merck R.G.) 10 ml. of $BF_3$-etherate was added, with vigorous swirling. After 3 min. the then deep blue solution was poured rapidly, with swirling, into 1200 ml. of 80% acetone. After the addition of 200 ml. of hexane

(8) L. Zechmeister and F. J. Petracek, THIS JOURNAL, **77**, 2567 (1955).

(9) E. M. Shantz, J. D. Cawley and N. D. Embree, *ibid.*, **65**, 901 (1943); P. Karrer and R. Schwyzer, *Helv. Chim. Acta*, **31**, 1055 (1948); P. Meunier *Compt. rend.*, **227**, 206 (1948).

the epiphase was washed in an automatic apparatus for 30 min., then dried with sodium sulfate and evaporated. The solution of the residue in 5 ml. of benzene was diluted with hexane to 50 ml. and developed with the latter solvent on a 27 × 8 cm. column. There appeared near the top a 70 mm. wide red-orange zone of the main product, *viz.*, 4-hydroxy-β-carotene (also containing some *cis* isomers), well separated from several minor zones. When the alcohol eluate of the main zone was transferred to hexane and rechromatographed (developer, hexane +5% acetone), it was resolved into an all-*trans* zone (yield, 32% determined photometrically) and a lighter zone containing some of the *cis* forms. The *trans* fraction was eluted. After evaporation of the hexane solution, crystallization from chloroform–ethanol yielded 18 mg. of oval plates, m.p. 166–168° (cor.).

(b) **From 3,4-Dehydro-β-carotene.**—This experiment was carried out with 20 mg. of substance as just described, but the time allowed for the complex formation was reduced to 1 min. The photometrically estimated yield was 2.5 mg. After recrystallization, the compound melted at 165–168°.

(c) **From *retro*-Dehydrocarotene.**—A similar treatment of 100 mg. of substance with 10 ml. of $BF_3$-etherate at 0° for 15 sec. resulted in the isolation of 13 mg. of the all-*trans* product, m.p. 166°.

These pure samples did not show either a m.p. depression with, or a chromatographic separation from, an authentic preparation of isocryptoxanthin (*ex retro*-dehydrocarotene). Partition behavior and the spectra (including the infrared region) were identical. The test for allylic hydroxyl was strongly positive.

*Anal.* Calcd. for $C_{40}H_{56}O$: C, 86.90; H, 10.21. Found: C, 87.08; H, 10.44 (this sample was prepared according to section a).

**4-Hydroxy-α-carotene.**—3,4-Dehydro-α-carotene (70 mg.) was treated with $BF_3$-etherate as described above in section b; yield 17.5 mg. (photometric estimation). Upon crystallization from benzene–methanol, 10 mg. of long, rectangular plates (mostly with broken ends) were obtained; m.p. 177° (sintering at 173°). The spectrum was identical with that of α-carotene; furthermore, no separation was observed on the column from an authentic sample prepared by Mr. W. V. Bush in our laboratory (unpublished). The test for allylic hydroxyl was positive.

*Anal.* Calcd. for $C_{40}H_{56}O$: C, 86.90; H, 10.21. Found: C, 87.01; H, 10.34.

**2-Hydroxy-3,4-dehydro-β-carotene from *retro*-Bisdehydrocarotene.**—To a solution of 50 mg. of crystalline all-*trans*-*retro*-bisdehydrocarotene[10] in 50 ml. of ethanol-free chloroform (0°), 5 ml. of $BF_3$-etherate was added, with swirling. Thirty seconds later the dark blue liquid was poured rapidly into 800 ml. of pre-cooled 75% acetone. The then orange solution was transferred without delay into a 1-liter separatory funnel containing 100 ml. of hexane. Water was added and the upper phase was washed thoroughly. After drying and evaporation, the red, oily residue was dissolved in 50 ml. of hexane and developed with hexane + 7% acetone on a 27 × 8 cm. column (the figures represent width of zones in mm.):

| | |
|---|---|
| 90 | several colored zones and interzones |
| 30 | pink-orange: all-*trans*-2-hydroxy-3,4-dehydro-β-carotene |
| 40 | several orange zones: *cis* isomers of the former |
| 90 | minor yellow zones in a mainly colorless section |

The 30-mm. zone was eluted, transferred to hexane, washed and dried; yield, 14.5 mg. (estimated photometrically). A solution containing 40 mg. of substance was developed with benzene + hexane (1:1) on two 27 × 8 cm. columns. From the combined main zones a powdery, red-orange residue was obtained that yielded, when crystallized from chloroform–methanol, 30 mg. of sturdy square prisms, m.p. 180–182°; partition behavior, 90:10 in hexane:95% methanol. The spectral curve was identical with that of 4-hydroxy-3',4'-dehydro-β-carotene; $E^{mol}_{1\,cm}$ 12.5 × $10^4$ at $\lambda_{max}$ 459 mμ.

*Anal.* Calcd. for $C_{40}H_{54}O$: C, 87.22; H, 9.88. Found: C, 87.36; H, 10.04.

In a mixed-chromatogram test (hexane + 7% acetone) the compound appeared below the 4-hydroxy isomer, from which it was clearly separated by a colorless interzone; reaction with HCl–chloroform, strongly positive.

**Methyl Ether.**—A solution of 9 mg. of the 2-hydroxy compound in 2 ml. of chloroform was diluted with 20 ml. of abs. methanol, whereupon 5 drops of HCl–chloroform were introduced. Three minutes later the liquid was diluted with 10 ml. of hexane, then washed, dried and evaporated. The hexane solution of the red, oily residue was adsorbed on a 24 × 2 cm. column and developed with hexane + 2% acetone. The main, orange zone contained all-*trans*-2-methoxy-3,4-dehydro-β-carotene, followed by a *cis* isomer (photometrically estimated total yield, 5.5 mg.).

The all-*trans* fraction gave irregular plates, m.p. 145°; partition behavior, 99:1 in hexane:95% methanol. The spectral curve in the visible and ultraviolet regions was identical with that of the unmethylated compound; reaction with HCl–chloroform, strongly positive.

***retro*-Bisdehydrocarotene from 2-Hydroxy-3,4-dehydro-β-carotene.**—The substance (5 mg.) was dehydrated in chloroform as described for the 4-hydroxy isomer[4,5] and the reaction product was resolved by developing with hexane + 8% acetone on a 24 × 4 cm. column. The main zone represented all-*trans*-*retro*-bisdehydrocarotene, followed by *cis* zones (total, 4 mg.). The all-*trans* isomer showed the expected spectrum (518, *487*, 460 mμ) and did not separate on the column from an authentic sample of the *retro* compound. No 3,4,3',4'-bisdehydro-β-carotene was formed in this reaction.

**The $BF_3$ Complex of Anhydrovitamin $A_1$ and its Hydrolysis.**—The starting material was prepared by treating 200 mg. of crystalline vitamin $A_1$ in 50 ml. of chloroform with 30 drops of the HCl–chloroform reagent until the peaks typical for the anhydro derivative (350, *369*, 390 mμ) did not show further increase (about 15 min.). After elimination of the acid by washing, drying and evaporation, a hexane solution of the residue was developed with hexane + 10% benzene on a 25 × 5 cm. alumina–lime–Celite column (3:1:1). The main fluorescent zone contained 38 mg. of the anhydro compound.[11]

To a solution of 150 mg. of anhydrovitamin $A_1$ in 50 ml. of alcohol-free chloroform (0°) 5 ml. of $BF_3$-etherate was added. After 10 sec. the blue complex was cleaved by pouring the solution into 500 ml. of 80% acetone (0°). After dilution with 100 ml. of hexane the epiphase was washed thoroughly, dried, evaporated and developed with hexane + 5% acetone on a 21 × 8 cm. column. Vitamin $A_1$ was absent.

| | |
|---|---|
| 15 | bright blue fluorescence in ultraviolet light |
| 45 | pale blue fl. |
| 40 | interzone |
| 7 | yellow fl. |
| 40 | interzone |
| 25 | blue fl.: 4-hydroxy-axerophthene |
| 40 | interzone |
| 5 | yellow fl. |

The 25-mm. zone was eluted with ethanol and transferred to hexane; yield 22 mg.; partition behavior, 62:38 in hexane:95% methanol. The adsorption affinity of the product was much inferior to that of vitamin A (lime–Celite; hexane + 5% acetone).

(10) Solutions of *cis* forms can also be used, without previous crystallization. The following *improved method for the preparation of retro-bisdehydrocarotene* takes advantage of the high yields (40%) of 4-keto-3',4'-dehydro-β-carotene available from β-carotene.[4,5] The unresolved mixture of the ketones thus formed is reduced with lithium aluminum hydride, whereupon the main product, *viz.* a mixture of *trans*- and *cis*-4-hydroxy-3',4'-dehydro-β-carotene, is separated from some minor constituents on lime–Celite (hexane + 7% acetone) and then dehydrated by means of HCl–chloroform to *cis–trans* isomeric *retro*-bisdehydrocarotenes (over-all yield, 24%; yield of the crystalline all-*trans* form, 7%).

(11) No unreacted vitamin A was observed on the column. Near the top, however, an intensely blue-fluorescent zone appeared whose spectrum (333, *348*, 367 mμ) and adsorption behavior indicated that it may well be the vitamin A isomer described by Cawley and Seidel (unpublished; *cf.* J. G. Baxter, *Fortschr. Chem. org. Naturstoffe*, **9**, 41 (1952), there p. 51). Its formation during the dehydration of vitamin $A_1$ has not been noted before, although it has recently been shown that vitamin $A_1$ acetate under similar conditions gives the acetate of the Cawley isomer. R. H. Beutel, D. F. Hinkley and P. I. Pollak, THIS JOURNAL, **77**, 5166 (1955), called this compound *retro*-vitamin A. The term *retro*-vitamin $A_1$ is preferable.

**Reconversion of 4-Hydroxy-axerophthene to Anhydrovitamin $A_1$.**—Four drops of the HCl–chloroform reagent were added to a solution of 8 mg. of the hydroxy compound in 10 ml. of alcohol-free chloroform. Within 5 min. the liquid turned yellow. It was then transferred (with the aid of 20 ml. of hexane) into a separatory funnel. After the addition of water the epiphase was washed, dried and evaporated. The solution of the oily residue in a few ml. of hexane was developed on a 20 × 2 cm. magnesia–lime–Celite column (3:1:1). Below some minor pale zones the strongly orange fluorescent main zone (5 mm. thick) of the anhydrovitamin appeared; yield 1.25 mg. Spectral curve and adsorption behavior (magnesia–lime–Celite 3:1:1, hexane) were identical with a sample obtained by dehydrating crystalline vitamin $A_1$.

**Acknowledgment.**—The microanalyses were carried out by Dr. A. Elek in Los Angeles and Mr. G. Swinehart in Dr. Haagen-Smit's laboratory in Pasadena.

PASADENA, CALIFORNIA

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[CONTRIBUTION FROM THE GENERAL RESEARCH ORGANIZATION, OLIN MATHIESON CHEMICAL CORP.]

# The Position Isomerism of the Oleic Acid Formoxylation Reaction

BY JACK ROCKETT

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The perchloric acid catalyzed addition of formic acid to oleic acid has been examined to determine the positions on the carbon chain to which the formoxy group becomes attached. *Via* a series of syntheses, a mixture of dibasic acids was obtained which, upon chromatographic separation, was shown to be a 50–50 molar mixture of azelaic and sebacic acids. The formic acid addition, therefore, takes place equally, and perhaps exclusively, at the 9- and 10-positions.

A recent paper[1] describes the perchloric acid catalyzed addition of formic acid to olefins. The addition to oleic acid resulted in a mixture of formoxystearic acids which, upon hydrolysis, gave a mixture of hydroxystearic acids of relatively high melting point. The ease with which 10-hydroxystearic acid could be obtained therefrom by repeated crystallizations led to the conjecture that the mixture must be essentially 9- and 10-hydroxystearic acids. The formoxylation of 1-hexene, however, gave, after hydrolysis, a mixture of 2- and 3-hexanol. No 1-hexanol could be found. The wandering of the point of addition in this reaction would lead one to suspect that the the formoxylation of oleic acid might have resulted in isomers other than 9- and 10-formoxystearic acids.

In order to determine the exact nature of the position isomers formed, the mixed hydroxystearic acids obtained as described by Knight, Koos and Swern,[1] was oxidized to a mixture of the corresponding keto-acids whose oximes were rearranged *via* the Beckmann rearrangement. The mixture of amides obtained thereby was hydrolyzed in an autoclave, and the hydrolysis products were separated by the method of Ross.[2] The mixture of dibasic acids was analyzed by elution chromatography through a silicic acid column.[3]

Three successive chromatograms were performed, giving an average value of 50.1 mole per cent. for sebacic acid and 49.9 mole per cent. for azelaic acid. A typical elution curve is shown in Fig. 1. Clearly two, and only two, dibasic acids were present in the mixture. The elution peaks were identified as sebacic and azelaic acids. The addition of formic acid to oleic acid takes place equally, therefore, at the 9- and 10-carbon positions. There appears to be no wandering from the site of the double bond.

In order to avoid loss of isomers, the entire

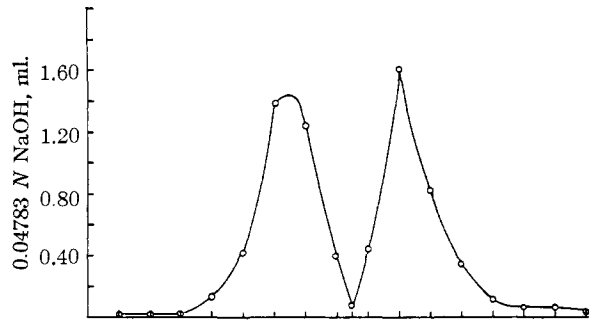


Fig. 1.—Titration of eluted samples from chromatographic column.

reaction product obtained in each step was used for the succeeding reaction. One recrystallization of ketostearic acid from acetone was found to be necessary in order to obtain a crystalline amide in the subsequent rearrangement. It is possible that isomers other than the 9- and 10-ketostearic acids were lost in this operation. The possibility of addition at positions other than $C_9$ and $C_{10}$, therefore, has not been completely eliminated. However, no trace of the existence of other isomers was found in the final mixture of dibasic acids.

It must be presumed that, although perchloric acid favors shifting of the carbonium ion from a primary to a secondary carbon atom (the 1-hexene addition), it does not cause any wandering of a secondary carbonium ion. This is in contrast to sulfuric acid which has been shown to do so.[4]

It is apparent, also, that there is neither an induced effect nor a field effect due to the carboxyl group at the end of the chain. Both 9- and 10-carbon atoms were perfectly equivalent in this reaction. This is in marked contrast to the hydrogenation of epoxystearic acid which is claimed to give only 10-hydroxystearic acid.[5]

(1) H. B. Knight R. E. Koos and D. Swern, THIS JOURNAL, **75**, 6212 (1953).

(2) J. Ross, A. I. Gebhart and J. F. Gerecht, *ibid.*, **71**, 285 (1949).

(3) T. Higuchi, N. C. Hill and G. B. Corcoran, *Anal. Chem.*, **24**, 491 (1952).

(4) B. B. Schaeffer, E. T. Roe, J. A. Dixon and W. C. Ault, THIS JOURNAL, **66**, 1924 (1944).

(5) C. H. Mack and W. G. Bickford, *J. Org. Chem.*, **18**, 686 (1953).