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VITAMIN A ANALOGUES—V*

SYNTHESIS OF 9-, 13-, AND 9,13- DESMETHYL HOMOLOGUES OF VITAMIN A

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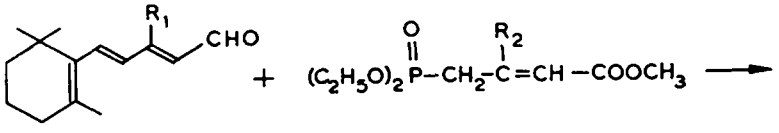
Abstract—The syntheses of 9-, 13-, and 9,13-desmethyl-vitamin A are described from the corresponding desmethyl homologues of vitamin A acid methyl esters. The latter were prepared by condensation of *trans*-β-ionylidene acetaldehyde and *trans*-5-(2′,6′,6′-trimethylcyclohexene-1′-yl-1′)-pentadiene 2,4-al-1 respectively, with the phosphonates of the appropriate crotonic acid esters. Biological investigations on growth promoting properties have shown that *all-trans*-13- and *all-trans*-9,13-desmethyl vitamin A acetate are completely inactive, while *all-trans*-9-desmethyl vitamin A acetate exhibits an activity of about 4%, when compared with *all-trans*-vitamin A acetate.

IN CONNECTION with our investigations‡ on the relationship between chemical structures and biological activity of vitamin A the 9-, 13-, and 9,13- desmethyl homologues of vitamin A acetate were synthesized.

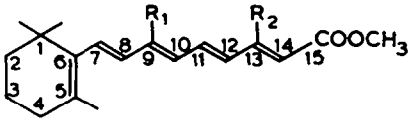
One of these homologues—the 13-desmethyl vitamin A—has been described earlier by Heilbron *et. al.*[1] These authors report a purity of about 25% for the very unstable product they obtained, the geometric structure of which was not determined.

The 9-, 13-, and 9,13- desmethyl homologues of vitamin A were prepared from the corresponding desmethyl homologues of vitamin A acid methyl esters by reduction with LAH or DIBAH at low temperature.

These esters were synthesized according to the following general reaction scheme:



Ia : R_1 = H IIa : R_2 = CH_3
Ib : R_1 = CH_3 IIb : R_2 = H



IIIa : R_1 = H ; R_2 = CH_3
IIIb : R_1 = CH_3 ; R_2 = H
IIIc R_1 = R_2 = H

* Part IV of this series: J. L. Baas, Mrs. A. Davies-Fidder and H. O. Huisman, *Tetrahedron* 22, 285 (1966).

† Part of the Thesis of P. J. van den Tempel, University of Amsterdam (1965).

‡ These investigations are carried out in collaboration with the Laboratories of N. V. Philips-Duphar, Weesp, The Netherlands.

[1] G. W. H. Cheeseman, I. M. Heilbron, E. R. H. Jones, F. Sondheimer and B. C. L. Weedon, *J. Chem. Soc.* 1526 (1949).

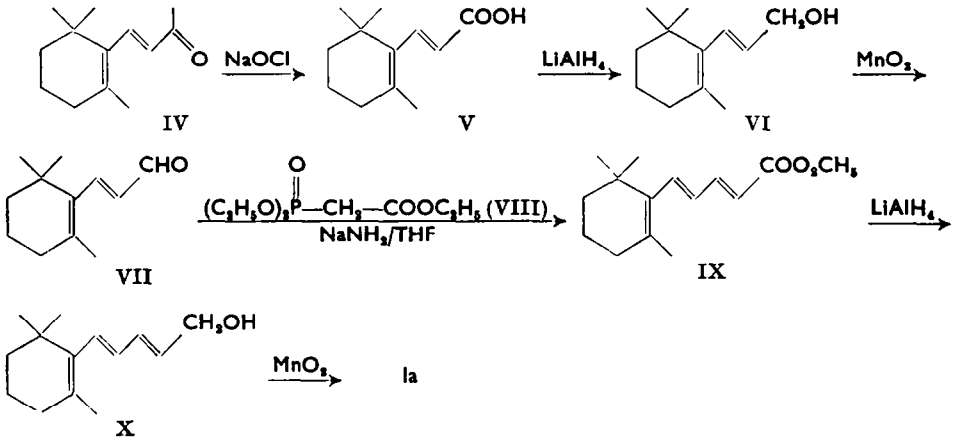
By means of the phosphonate carbanion variation[2] of the Wittig-reaction, using the favourable experimental conditions described by Takahashi,[3] 5-(2',6',6'-trimethyl-cyclohexene-1'-yl-1')-pentadiene-2,4-al-1 (Ia), and β-ionylidene acetaldehyde (Ib) were condensed with the γ-phosphonates of β-methylcrotonic acid methyl ester (IIa) and crotonic acid methyl ester (IIb).

The *all-trans*- 13- (IIIb) and *all-trans*- 9,13-desmethyl vitamin A acid methyl ester (IIIc) were isolated as pure crystalline compounds in yields of 70–80%.

The 9-desmethyl vitamin A acid methyl ester (IIIa) was obtained as an oil from which the corresponding pure crystalline *all-trans*- acid was prepared by hydrolysis.

The phosphonates (IIa and IIb) were prepared by means of an Arbuzov reaction[4] between triethylphosphite and γ-bromo-β-methylcrotonic acid methyl ester and γ-bromocrotonic acid methyl ester respectively.

The aldehyde Ia was synthesized using the following reaction schemes:



The *all-trans* structure of IX was deduced by comparing the NMR spectra of IX (Fig. 2) and *trans*-β-ionylidene acetic acid methyl ester (Fig. 1).

In these two NMR spectra the following characteristic differences are observed:

1. Instead of the *singlet* from the ester methyl protons (Fig. 1) ($\delta = 3{\cdot}68$) a *triplet* ($\delta = 1{\cdot}25$) from the methyl- and a *quartet* ($\delta = 4{\cdot}14$) from the methylene protons of the ester ethyl group.

Integration made it clear that (Fig. 1) three protons absorb in the region of the field between 405 and 355 c/s lower than the reference signal.

2. H(10) gives a *doublet* (due to the influence of H(9)) with a chemical shift $\delta = 5{\cdot}75$ instead of the *singlet* $\delta = 5{\cdot}75$ in Fig. 1. $J_{H(10)H(9)} = 16$ c/s, indicating a *trans* double bond between C_9 and C_{10}.

3. In the spectra of *trans* β-C_{15}-ester, H(8) is found as a *doublet* (coupling with H(7) and H(9)); $\delta = 6{\cdot}12$. $J_{H(8)H(7)} = 16$ c/s, *trans* double bond between C_7 and C_8.

4. The *quartet* in Fig. 1 ($\delta = 7{\cdot}26$) must be ascribed to H(9) coupling with H(8) and H(10), $J_{H(9)H(10)} = 16$ c/s.

[2] W. S. Wadsworth and W. D. Emmons, *J. Amer. Chem. Soc.* **83**, 1733 (1961).
[3] H. Takahashi, *Bull. Chem. Soc., Japan* **15**, 1498 (1962).
[4] G. M. Kosolapoff, *Organophosphorous Compounds* J. Wiley, N.Y. Chap. 7 (1950).

The stereochemistry of 9-desmethyl vitamin A acid and 13- and 9,13-desmethyl vitamin A acid methyl ester was deduced from a comparison of the UV and NMR spectra of these compounds with the corresponding spectra of *all-trans* vitamin A acid methyl ester.* (Figs 3, 4 and 5).

The stereochemistry of the starting materials (aldehydes and phosphonates) is

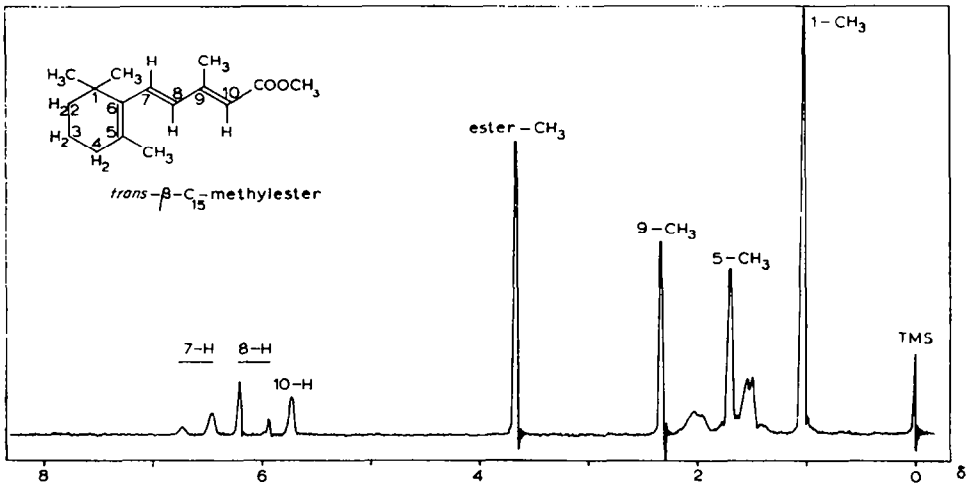


FIG. 1

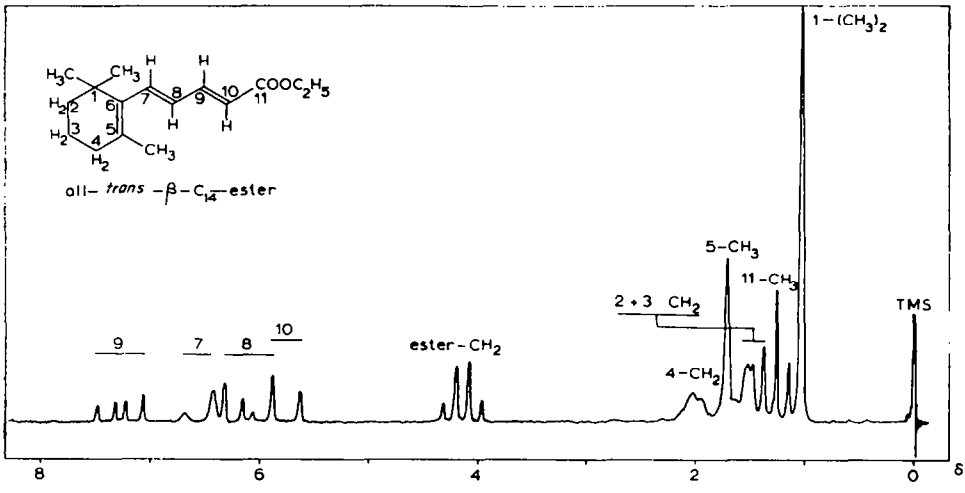


FIG. 2

known to be *all-trans*. This fact, combined with a comparison between the respective NMR spectra, leaves no doubt about the stereochemistry of the acid (Fig. 3) and the esters (Figs 4 and 5), except on one point, namely, that a *cis*-configuration for the C_{11}—C_{12} double bond can not be excluded. However, a *cis*-position of the protons on the C_{11}—C_{12} double bond, more than on any other, gives rise to a substantial

* Compare also Part III of this series: P. K. Korver, C. Kruk, P. J. van der Haak, J. L. Baas and H. O. Huisman, *Tetrahedron* **22**, 277 (1966).

decrease in the intensity of the major peak in the UV absorption spectra of all vitamin A derivatives (*all-trans*-vitamin A: $\varepsilon = 52{,}480$; 11-*cis*: $\varepsilon = 39{,}400$,[5] (*all-trans*-vitamin A acetate: $\varepsilon = 51{,}180$; 11-*cis*: $\varepsilon = 31{,}960$,[6] (*all-trans*-vitamin A aldehyde: $\varepsilon = 43{,}400$; 11-*cis*: $\varepsilon = 24{,}900$).[5]

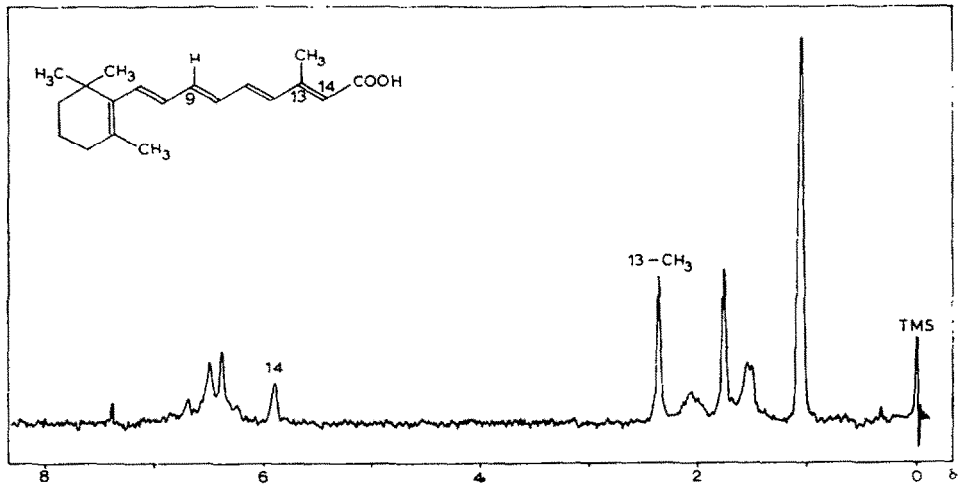


FIG. 3. *All-trans* 9-desmethyl vitamin A acid

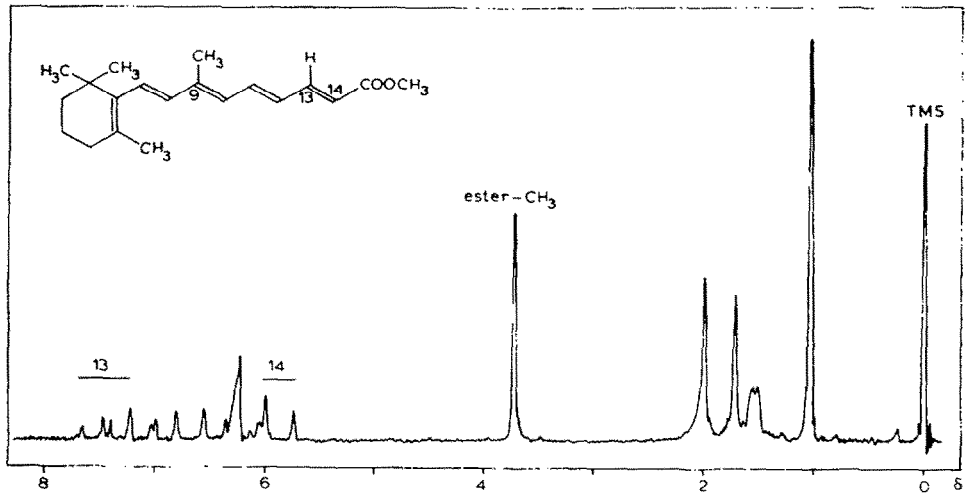


FIG. 4. *All-trans* 13-desmethyl vitamin A acid methyl ester

From Tables 1 and 2 it is seen that no comparable decrease in intensity is found in the UV spectra of the synthesized homologous vitamin A acid and vitamin A acid esters.

The acetates were prepared by reduction with LAH of the corresponding esters (the methyl ester of *all-trans* 9-desmethyl vitamin A acid was prepared from the pure acid with diazomethane) followed by treatment of the crude vitamin A

[5] P. K. Brown and G. Wald, *J. Biol. Chem.* **222**, 865 (1956).

[6] M. Kofler and S. G. Rubin, *Vitamins and Hormones* Vol. 18; p. 325 (1960).

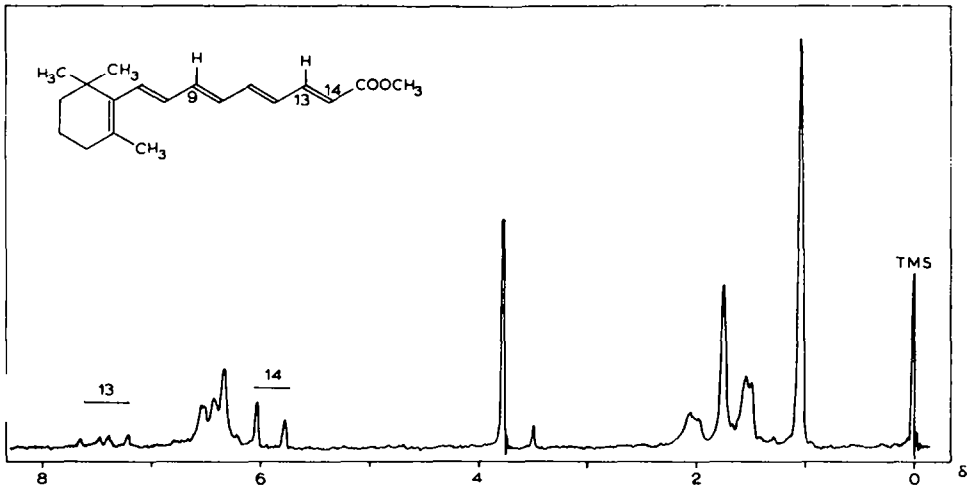


FIG. 5. *All-trans* 9,13-desmethyl vitamin A acid methyl ester

homologues with acetyl chloride.[7] No isomerization took place under the applied experimental conditions.

TABLE 1

	λ_{max}	ε
All-trans vitamin A acid methyl ester	350 nm	45,200
All-trans 9-desmethyl vitamin A acid	346 nm	41,700
All-trans 13-desmethyl vitamin A acid methyl ester	354 nm	42,000
All-trans 9,13-desmethyl vitamin A acid methyl ester	350 nm	43,100

TABLE 2

	λ_{max}	ε
All-trans vitamin A acetate	326 nm	51,180
All-trans 9-desmethyl vitamin A acetate	321 nm	43,000
All-trans 13-desmethyl vitamin A acetate	326 nm	46,000
All-trans 9,13-desmethyl vitamin A acetate	320 nm	46,500

Biological experiments

Biological investigations on the growth promoting properties with chickens show that *all-trans* 13- and *all-trans* 9,13-desmethyl vitamin A acetates are completely inactive, while *all-trans* 9-desmethyl vitamin A acetate exhibits an activity of about 4% when compared with *all-trans* vitamin A acetate.

EXPERIMENTAL

The IR spectra were measured on a Unicam SP 200 Spectrophotometer and the UV spectra on a Zeiss RPQ 20 C self-recording Spectrophotometer.

The NMR spectra were obtained with a Varian A 60 Analytical Spectrometer. The compounds were measured as 10% solutions in CCl_4. Chemical shifts are given from tetramethylsilane as in internal reference. The spectrometer calibration was checked by the procedure given by Jungnickel.[8]

For detailed informations of the interpretations of some NMR spectra compare also part III of this series.[9]

[7] H. O. Huisman, A. Smit, P. H. van Leeuwen and J. H. van Rij, *Rec. Trav. Chim.* **75**, 977 (1956).
[8] J. L. Jungnickel, *Analyt. Chem.* **35**, 985 (1963).
[9] P. K. Korver, C. Kruk, P. J. van der Haak, J. L. Baas and H. O. Huisman, *Tetrahedron* **22**, 277 (1966).

M.ps were determined with a Kofler microscope. Both m.ps and b.ps are uncorrected. All experiments were carried out in a N_2 atm.

3-(2′,6′,6′-*Trimethylcyclohexene*-1′-*yl*-1′)-*propenoic acid* (V). Chlorine (0·3 mole) was led into a solution of 1 mole NaOH in 100 ml water (solution kept at 0°). This solution (containing 0·255 mole NaOCl) was added with stirring to 16 g (0·185 mole) IV, kept at 0°; then 20 ml MeOH were carefully added. The reaction mixture was acidified with H_3PO_4. The precipiated acid was dissolved in 10% NaOHaq. The resulting solution of the Na-salt of V was extracted with ether to remove any unreacted β-ionon.

Acidification of the alkaline solution with H_3PO_4 followed by extraction of the aqueous solution with ether and evaporation of the solvent gave a residue which was recrystallized from 60% EtOH. The acid (V) was obtained in 80% yield (lit.[10]) 75% on crude residue), m.p. 105·5–108°. UV absorption spectrum: λ_{max} 279 nm; $\varepsilon = 9{,}700$ (lit. λ_{max} 277 nm; $\varepsilon = 9{,}240$).

3-(2′,6′,6′-*Trimethylcyclohexene*-1′-*yl*-1′)-*propene*-2-*ol*-1 (VI). A solution of 70 g (0·36 mole) V in 100 ml abs. ether was added slowly to a suspension of 15 g (50% in excess) LAH in 100 ml abs. ether. After the addition was complete, the mixture was refluxed during 2 hr. The complex was decomposed by the addition of a sat. NH_4Claq. The precipitate was filtered off and the resulting filtrate dried over $MgSO_4$. After removal of the solvent the residue was distilled *in vacuo*, yield 50 g (70%) VI; b.p. 78–80°/0·3 mm; n_D^{20}: 1·5078; UV absorption spectrum: λ_{max} 234 nm; $\varepsilon = 6{,}300$.

3-(2′,6′,6′-*Trimethylcyclohexene*-1′-*yl*-1′)-*propene*-2-*al*-1 (VII). Compound VI (40 g) was stirred with 400 g MnO_2 in 1 l. CCl_4 under N_2. The IR spectra of samples taken from the reaction mixture showed that the alcohol was completely oxidized to the aldehyde VII within ½ hr. The MnO_2 was filtered off and the solvent evaporated. Distillation of the residue *in vacuo* yielded 35 g (87%) of VII; b.p. 66–67°/0·2 mm; n_D^{20}: 1·5380; UV absorption spectrum: λ_{max} 295 nm; $\varepsilon = 10{,}500$; VII was identified as the 2,4-dinitrophenylhydrazone; m.p. 180–181°. (Found: C, 60·31; H, 6·19; N, 15·50. Calc. for $C_{16}H_{22}O_4N_4$ (358·4): C, 60·32; H, 6·19; N, 15·61%.)

trans-1-*Carbethoxy*-4-(2′,6′,6′-*trimethylcyclohexene*-1′-*yl*-1′)-*butadiene*-1,2 (IX). To a well-stirred suspension of 16 g (0·41 mole) $NaNH_2$ in 300 ml THF, 99 g (0·49 mole) VIII was added. The temp of the reaction mixture rose to about 55° during the addition. The mixture was kept at 60° for a further 1·5 hr. After cooling the mixture to 0°, 36 g (0·20 mole) VII was slowly added. The reaction mixture was kept at 40–50° for 2 hr. To the cooled mixture (0°) a sat. NaClaq was added. Extraction of the reaction mixture with pet. ether (b.p. 60–80°) was followed by drying of the extract over $MgSO_4$. The solvents were removed and the residue distilled *in vacuo*, yield 42 g (84%) of IX; n_D^{20}: 1·5483; b.p. 121–123°/0·45 mm. Compound IX crystallized in the refrigerator. After two recrystallizations from 80% EtOH, an analytically pure sample was obtained; m.p. 41·5–42°. UV absorption spectrum: λ_{max} 309 nm; $\varepsilon = 17{,}700$; λ_{max} 257 nm; $\varepsilon = 11{,}700$. (Found: C, 77·14; H, 9·69. Calc. for $C_{16}H_{24}O_2$ (222·3): C, 77·34; H, 9·71%.)

trans-5-(2′,6′,6′-*Trimethylcyclohexene*-1′-*yl*-1′)-*pentadiene*-2,4-*ol*-1 (X). A solution of 10 g (0·04 mole) IX in 20 ml abs. ether was slowly added to a stirred suspension of 1·5 g (0·04 mole) LAH in 80 ml abs. ether, kept at −5/+5°. After stirring the mixture for 2 hr at room temp the complex was decomposed by adding sat. NH_4Claq. The precipitate was filtered off and the filtrate dried over $MgSO_4$. Removal of the solvent was followed by distillation *in vacuo*; b.p. 108·5–109/0·15 mm; n_D^{20}: 1·5458, yield 6 g (70%).

trans-5-(2′,6′,6′-*Trimethylcyclohexene*-1′-*yl*-1′)-*pentadiene*-2,4-*al*-1 (Ia). Compound X (5 g) was oxidized with MnO_2 in CCl_4 at 60°; reaction time 10 min, yield 4·2 g (84%) Ia; b.p. 111–113°/0·4 mm: n_D^{20}: 1·5939. UV absorption spectrum: λ_{max} 265 nm; $\varepsilon = 13{,}500$; λ_{max} 327 nm; $\varepsilon = 19{,}300$. Compound Ia was identified as the 2,4-dinitrophenylhydrazone; m.p. 211–212°. (Found: C, 62·57; H, 6·52; N, 14·47. Calc. for $C_{20}H_{24}O_4N_4$ (384·4): C, 62·49; H, 6·29; N, 14·57%.)

1-*Carbomethoxy*-2-*methyl*-*propene*-1-*diethylphosphonate*-3 (IIa). Equimolecular quantities of triethylphosphite and γ-bromo-β-methylcrotonic acid methyl ester (prepared as γ-bromo-crotonic acid methyl ester[11]) were heated in a round bottomed flask. At a temp of about 120° a rather vigorous reaction took place. The mixture was kept at 120° for an additional hr. Distillation *in vacuo* yielded IIa in 77%; b.p. 118–120°/0·55 mm.

9-*Desmethyl vitamin A acid methyl ester* (IIIa). A mixture of 4 g (0·02 mole) Ia and 5·5 g (0·22 mole) IIa in 20 ml THF were added during a period of 15 min to a well-stirred suspension of 0·8 g

[10] W. G. Young, L. J. Andrews and S. J. Cristol, *J. Amer. Chem. Soc.* **66**, 520 (1944).
[11] A. I. Vogel, *Pract. Org. Chemistry* (3rd Edition) p. 927.

(0·02 mole) $NaNH_2$ in 30 ml THF, kept at −5°. After the addition was complete, the mixture was kept at 0° for another 30 min and then heated at 40–50° during 15 min. After cooling the mixture to 0° sat. NaClaq was added. Extraction with pet. ether (b.p. 60–80°) and drying of the extract was followed by removal of the solvents yielding a residue of 5·3 g (70%) yellow oil. UV spectrum: λ_{max} = 348 nm; ε 36,000.

All-trans-9-*desmethyl vitamin A acid.* Impure IIIa (2·5 g) was hydrolysed by boiling with a solution of KOH in dil. EtOH. The mixture was extracted with ether. The aqueous solution was carefully neutralized with 10% H_2SO_4. Extraction with ether was followed by drying of the ethereal extract over $MgSO_4$. After removal of the solvent a crystalline residue was obtained; 2·2 g (=90%). After 3 recrystallizations from EtOH the pure acid was isolated; m.p. 167–168°. UV spectrum: λ_{max} 346 nm; ε 41,700. (Found: C, 79·37; H, 9·02. Calc. for $C_{19}H_{26}O_2$ (284·4): C, 79·68; H, 9·15%.)

All-trans-9-*desmethyl vitamin A acetate.* Compound IIIa (prepared by reaction of the pure acid with diazomethane) was reduced with LAH at −5° in an ethereal solution. The complex was decomposed with dil. NaOHaq at 0°. The reaction mixture was treated in the usual way.

The rather unstable 9-desmethyl vitamin A was not isolated but immediately treated with a solution of acetyl chloride and pyridine in benzene at 0°. The acetate was obtained as a viscous oil. UV spectrum: λ_{max} 321 nm; ε 43,000.

All-trans-13-*desmethyl vitamin A acid methyl ester* (IIIb). A solution of 4·3 g (0·18 mole) *trans*-β-ionylidene-acetaldehyde and 5 g (0·021 mole) IIb in 20 ml THF was added in 15 min to a stirred suspension of 0·8 g (0·02 mole) $NaNH_2$ in 30 ml THF. The reaction was carried out at about 0°. The reaction mixture was kept at 0° for an additional 30 min and then for another 30 min at 30–40°. Sat. NaClaq was added to the reaction mixture cooled at 0°. Extraction with pet. ether (b.p. 60–80°) and drying of the extract was followed by removal of the solvents. The residue was recrystallized from 80% EtOH, yield: 4·5 g (75%); m.p. 99–100·5°. UV spectrum: λ_{max} 354 nm; ε 42,000. (Found: C, 79·99; H, 9·51. Calc. for $C_{20}H_{28}O_2$ (300·4): C, 79·95; H, 9·39%.)

All-trans-13-*desmethyl vitamin A acetate.* This acetate was prepared from IIIb in the way described above for the 9-desmethyl homologue. It was isolated as a viscous oil. UV spectrum: λ_{max} 326 nm; ε 46,000.

All-trans-9,13-*desmethyl vitamin A acid methyl ester* (IIIc). This compound was prepared as IIIb from the aldehyde Ia and the phosphonate IIb, yield 80%; m.p. 104–105°. UV spectrum: λ_{max} 350 nm; ε 43,100. (Found: C, 79·56; H, 9·13. Calc. for $C_{19}H_{28}O_2$ (286·4): C, 79·68; H, 9·15%.)

All-trans-*desmethyl vitamin A acetate.* Reduction of IIIc with Dibah (di-isobutylaluminium-hydride) immediately followed by treatment of the quantitatively formed 9,13-desmethyl vitamin A with acetyl chloride in a solution of pyridine and benzene yielded *all-trans*-9,13-desmethyl vitamin A acetate as a viscous oil in nearly 100% yield. UV spectrum: λ_{max} 320 nm; ε 46,500.

Comparison of the extinction values for the abs. maxima in the UV spectra of the esters (Table 1) with those of the corresponding acetates (Table 2) seems to justify estimated values for the purity of the different acetates of more than 85% for the *all-trans*-9-desmethyl vitamin A acetate and of more than 95% for the *all-trans*-13- and the *all-trans*-9,13-desmethyl homologues.

CONCLUSION

In view of the total lack of growth-promoting properties of the *all-trans*- 13- and 9,13-desmethyl vitamin A acetates and the very small activity (4% when compared with *all-trans*-vitamin A acetate) found for the *all-trans*- 9-desmethyl vitamin A acetate it may be concluded that the presence of *both* methyl groups on the side-chain of the *all-trans*- system are essential as far as growth promoting properties are concerned.

Acknowledgements—Thanks are due to Drs. P. J. van der Haak, Drs P. K. Korver, Mr. C. Kruk and Mr. M. P. F. van Noort of our Spectrometry Department for valuable assistance for measuring and interpreting the UV, IR and NMR spectra and to Mr. H. Pieters, Mr. W. J. Buis and Mr. J. F. van Duyl of our Microanalytical Department for carrying out the elementary analyses.

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