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VITAMIN A ANALOGUES—II*

SYNTHESIS OF 4-THIA-VITAMIN A

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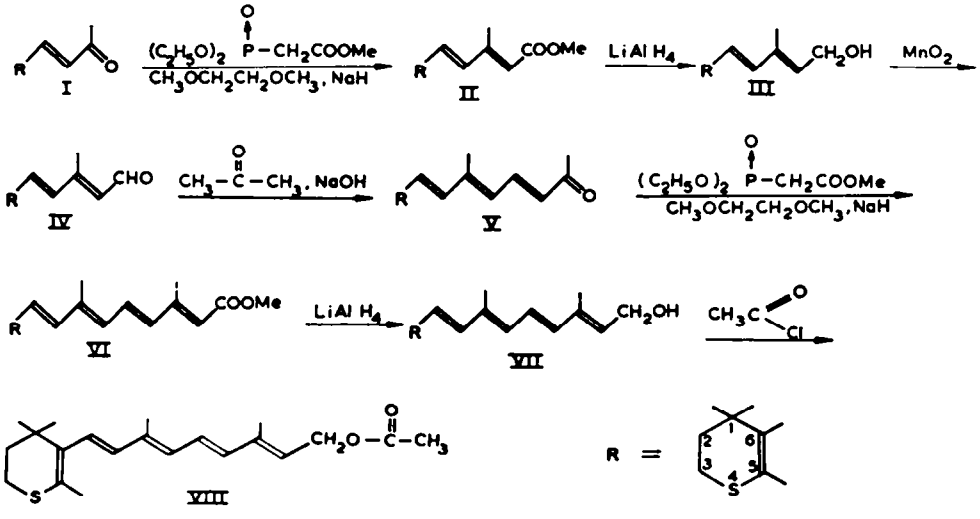
Abstract—The synthesis of 4-thia-vitamin A is described starting from 4-thia-ionone.[1] The stereochemical configurations of the intermediates obtained in the synthesis have been assigned by means of NMR spectrometry.[2] The UV absorption spectra of compounds of the 4-thia-vitamin A series show significant differences compared with the corresponding analogues of the vitamin A series. In biological growth test with chickens the *all-trans* 4-thia-vitamin A acetate shows ca. 5% of the activity of the *all-trans* vitamin A acetate.

IN CONNECTION with our investigations§ on the relationship between chemical structure and biological activity of vitamin A, 4-thia-vitamin A (VII) has been synthesized starting from 4-thia-ionone (I).[1]

The synthesis was carried out according to the following scheme: ||



I II III IV V VI VII VIII

* Part I: J. L. Baas, Mrs. A. Davies-Fidder and H. O. Huisman, *Tetrahedron* **22**, 259 (1966).

† Part of the Thesis of J. L. Baas, University of Amsterdam 1964.

‡ Part of the lecture presented at the IUPAC International Symposium on the Chemistry of Natural Products, Kyoto, Japan 12–18th April 1964.

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

|| In the scheme only the *all-trans* structures are given.

[1] See part I of this series: J. L. Baas, Mrs. A. Davies-Fidder, F. R. Visser and H. O. Huisman, *Tetrahedron* **22**, 259 (1966).

[2] See 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).

Methyl 4-thia-ionylidene acetate (II)

The methyl 4-thia-ionylidene acetate was obtained in a yield of 98% by reacting 4-thia-ionone and methyl diethyl phosphono acetate in dimethoxyethane using NaH as a base. It proved necessary to react *two* moles phosphonate with *one* mole of 4-thia-ionone in order to get the reported yields.

The methyl 4-thia-ionylidene acetate obtained consisted of a mixture of the *trans* and *cis* isomers in a ratio of ca. 75:25 as could be determined by means of NMR spectrometry.[2]

After converting the esters into the corresponding 4-thia-ionylidene acetic acids by saponification, the pure *trans* 4-thia-ionylidene acetic acid could be isolated by fractional crystallization. The mother liquor, obtained after filtering off the *trans*-acid consisted mainly of the *cis* isomer. However we were unable to isolate the *cis*-acid in pure crystalline form.

The pure *trans*-4-thia-ionylidene acetic acid was converted into the methyl ester by diazomethane. The correct geometric configuration of the ester was proved by NMR spectrometry.[2]

trans 4-*Thia-ionylidene ethanol* (III)

Reduction of the *trans* ester (II) into the corresponding *trans* 4-thia-ionylidene ethanol was carried out with LAH in ether at 0° in almost quantitative yield. The structure was proved by IR and NMR spectrometry. Attempts to characterize the alcohol as its crystalline anthraquinone-β-carboxylate were unsuccessful.

trans 4-*Thia-ionylidene acetaldehyde* (IV)

trans-4-thia-ionylidene acetaldehyde was obtained by oxidizing the corresponding *trans* alcohol by means of MnO_2 in petroleum ether (40–60°).[3,4] During the oxidation reaction no *trans* → *cis* isomerization occurred. NMR spectrum: *trans* $C_{(9)}CH_3$ δ = 2·29; *cis* $C_{(9)}CH_3$ δ = 2·10. The crude aldehyde was converted into its crystalline N,N-dimethylglycine hydrazone. During this conversion—according to a method given by Viscontini[5]—*trans* → *cis* isomerization partly took place for after regeneration in ethanol with HCl, the aldehyde was obtained as a mixture of the *trans* and *ics*- isomers.[2] A similar isomerization process has also been observed by van Leeuwen in the vitamin A series.[6]

The *trans* isomer of the N,N-dimethylglycine hydrazone of the 4-thia-ionylidene acetaldehyde was isolated in pure form by fractional crystallization. By regeneration of the hydrazone the pure *trans* aldehyde was obtained; no isomerization occurred during the regeneration process.

4-*Thia-ionylidene ethylideneacetone* (V)

The 4-thia-ketone (V) was synthesized by condensation of the *trans* 4-thia-ionylidene acetaldehyde with acetone. The crude ketone was purified via the crystalline N,N-dimethylglycine hydrazone. During the conversion into the

[3] S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J.* **42**, 516 (1948).
[4] H. O. Huisman, A. Smit, S. Vromen and L. G. M. Fisscher, *Rec. Trav. Chim.* **71**, 899 (1952).
[5] M. Viscontini and J. Meyer, *Helv. Chim. Acta* **33**, 1773 (1950).
[6] P. H. van Leeuwen, Thesis Amsterdam (1960).

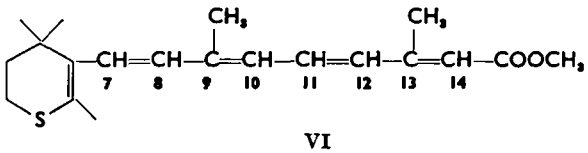
hydrazone *trans* → *cis* isomerization again partly took place. Since we were unsuccessful in isolating the pure *trans* isomer by fractional crystallization, a mixture of the *trans* and *cis* 4-thia-ionylidene-ethylidene acetone (in a ratio of ca. 70:30) was obtained after regeneration of the hydrazones in ethanol with HCl.

All trans 4-*thia-vitamin acid methyl-ester* (VI)

The condensation reaction of 4-thia-ketone (V) and methyl diethyl phosphono acetate was carried out under the same reaction conditions as described for the 4-thia-ionone.

Since the reaction was carried out with a mixture of the *trans* and the *cis* 4-thia-ketone (V), one could expect to obtain a mixture of four geometric isomers of 4-thia-vitamin acid methyl esters VI:

9-*trans*-13-*trans*
9-*trans*-13-*cis*
9-*cis*-13-*trans*
9-*cis*-13-*cis*



VI

The reaction mixture was hydrolysed and the *all-trans* 4-thia-vitamin A acid isolated by fractional crystallization.

The pure crystalline *all-trans* 4-thia-vitamin A acid was converted into the methyl ester by diazomethane in ether. The *all-trans* structure of the methyl ester was proved by NMR spectrometry.[3]

All-trans 4-*thia-vitamin* A (VII)

The *all-trans* 4-thia-vitamin A acid methyl ester was reduced by LAH in ether at low temperature and the *all-trans* 4-thia-vitamin A was isolated in an almost quantitative yield. The 4-thia-vitamin A is a rather unstable substance which must be stored under nitrogen at low temperatures; m.p. 36–37°. Attempts to convert the 4-thia-vitamin A into its crystalline anthraquinone-β-carboxylate derivative failed.

All-trans 4-*thia-vitamin* A *acetate* (VIII)

The unstable alcohol (VII) was converted into the more stable *all-trans* 4-thia-vitamin A acetate with acetyl chloride in pyridine. The acetate could be obtained in a crystalline form at low temperatures; at room temperature it is an oil.

The NMR spectrum of the *all-trans* 4-thia-vitamin A acetate was in complete agreement with the expected structure and showed a great similarity with the spectrum of the *all-trans* vitamin A acetate, particularly in respect to the absorptions of the side chain protons (Figs 1 and 2).

trans-4-*Thia-ionylidene cyanoacetic acid* (IX)

In connection with the significant differences between the UV absorption spectra of the compounds of the 4-thia-vitamin A series compared with the corresponding analogues of the vitamin A series we also synthesized 4-thia-ionylidene cyanoacetic acid from 4-thia-ionone (I) and cyanoacetic acid by means of the Knoevenagel condensation.[4]

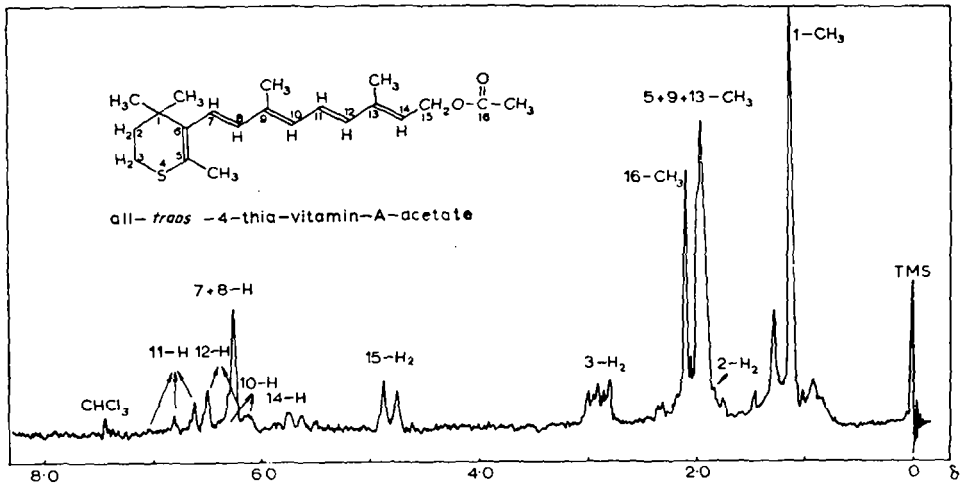


FIG. 1

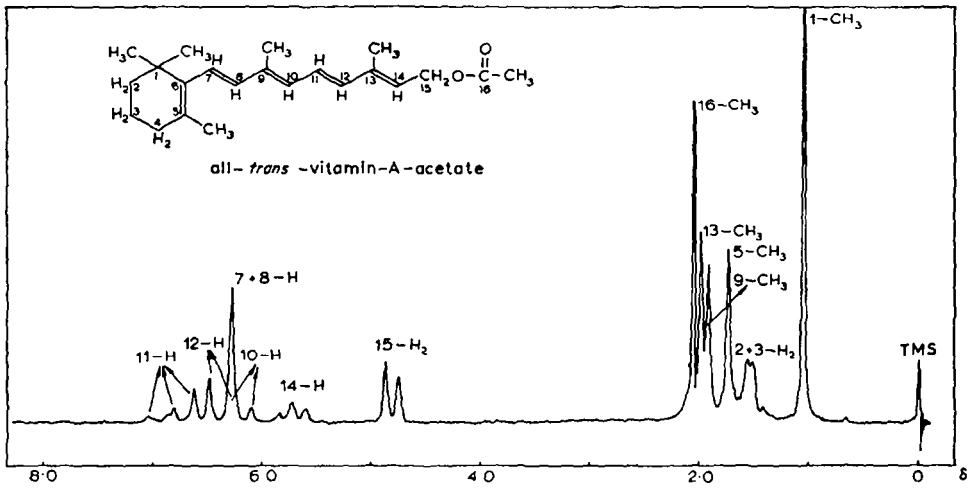
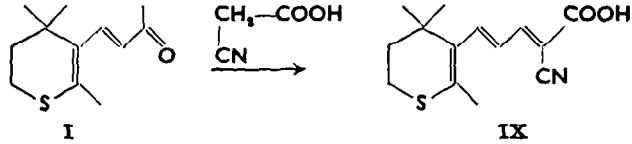


FIG. 2

From the crude reaction mixture the pure *trans* 4-thia-ionylidene cyanoacetic acid was isolated by fractional crystallization.



I IX

UV spectra

The UV absorption spectra of the 4-thia-vitamin A intermediates show remarkable differences in comparison with the corresponding analogues of the vitamin A series. The sulphur atom at position 4 seems to have a strong influence on both the geometric

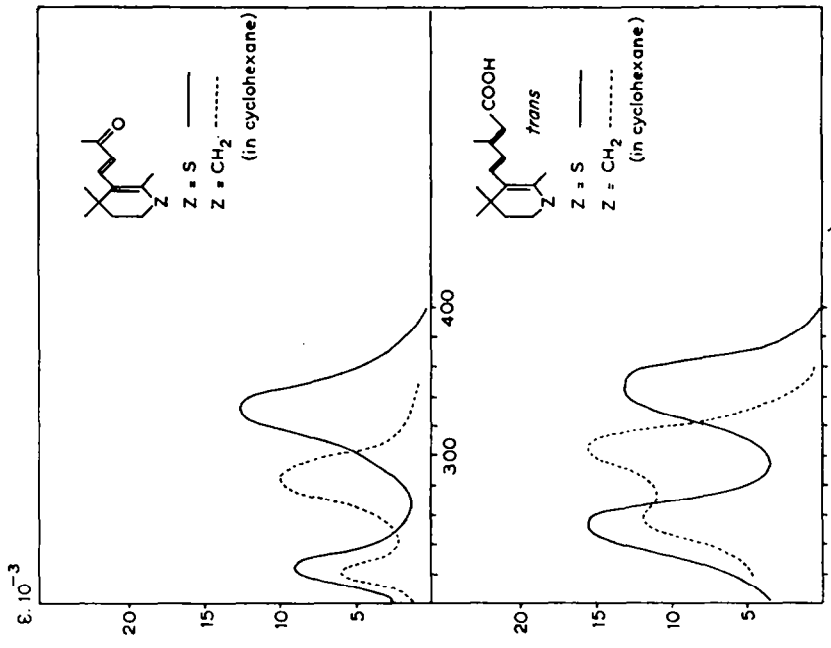


Diagram 1

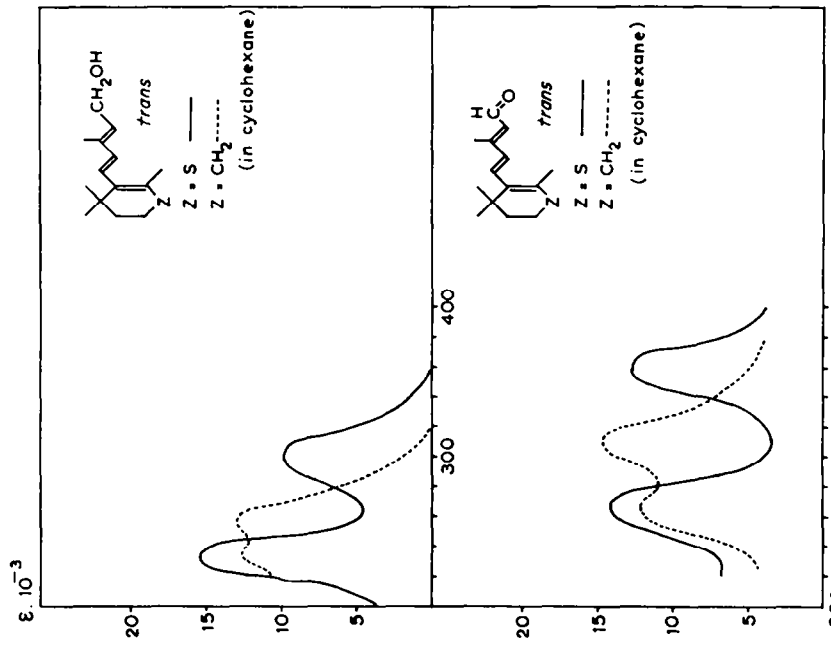


Diagram 2

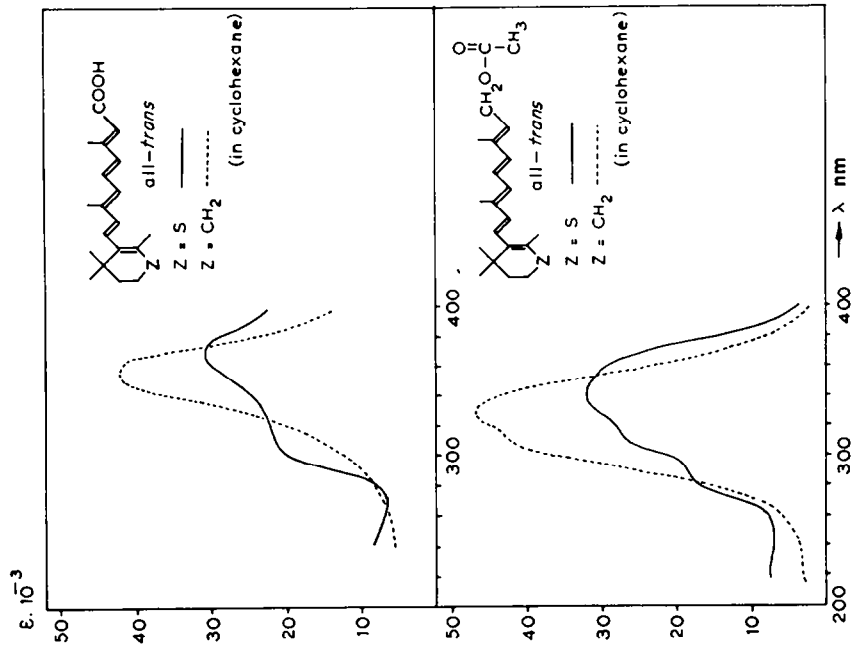


Diagram 4

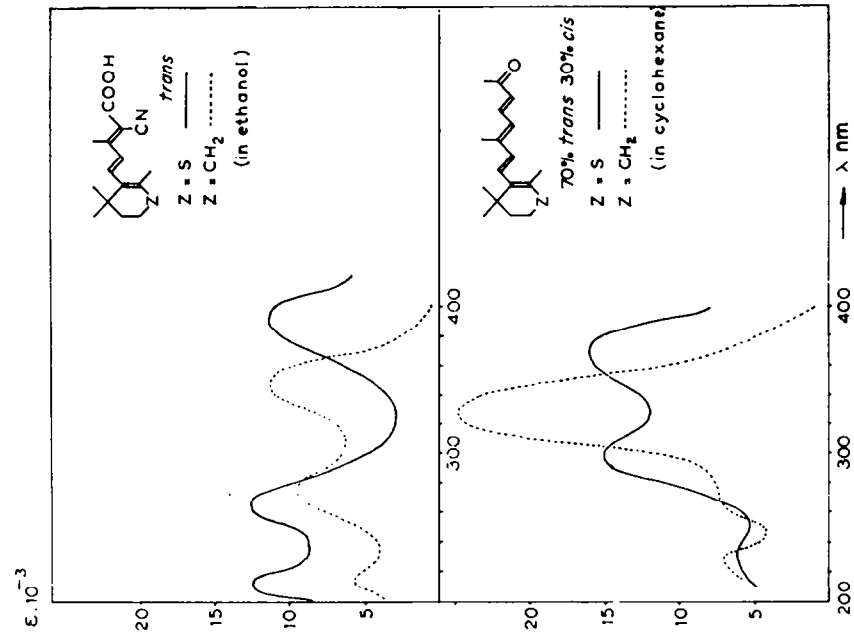


Diagram 3

TABLE I

4-Thia-vitamin A series			Vitamin A series		
Compound	Max (n.m.)	ε mole	Compound	Max (n.m.)	ε mole
4-Thia-ionone	223 333	9,000 12,800	β-ionone	221 284	7,000 10,000
trans-4-Thia-ionylidene acetic acid	255 349	15,600 13,100	*trans*-β-Ionylidene acetic acid	258 304	11,800 15,400
trans-Methyl 4-thia-ionylidene acetate	253 342	17,100 14,000	*trans*-β-Ionylidene acetate	258 299	11,900* 15,300
trans-4-Thia-ionylidene ethanol	233 300	15,500 9,600	*trans*-β-Ionylidene ethanol	235 255	12,300 12,800
trans-4-Thia-ionylidene acetaldehyde	266 360	14,300 13,000	*trans*-β-Ionylidene acetaldehyde	267 310	12,200 14,600
4-Thia-ionylidene ethylidene acetone (70% *trans*, 30% *cis*)	299 368	15,000 16,000	β-Ionylidene ethylidene acetone (70% *trans*, 30% *cis*)	267 327	7,500 25,000
All-trans-4-thia-vitamin A acid	310 367	21,000 31,000	*All-trans*-vitamin A acid	357	42,400
All-trans-4-vitamin A acid methylester	310 363	18,500 30,000	*All-trans*-vitamin A acid methylester	350	45,200*
All-trans-4-thia-vitamin A acetate	296 310 334	19,500 26,000 31,000	*All-trans*-vitamin A acetate	328	48,000
trans-4-Thia-ionylidene cyano-acetic acid*	265 395	12,900* 11,500*	*trans*-β-Ionylidene cyano-acetic acid	272 342	9,500* 11,200
N,N-Dimethylglycine hydrazone of: 4-Thia-ionone	257 322	14,200 17,000	N,N-Dimethylglycine hydrazone of: β-Ionone	211 285	12,700 19,300
4-Thia-ionylidene acetaldehyde	277 345	15,500 17,800	*trans*-Ionylidene acetaldehyde	327	41,800*
4-Thia-ionylidene ethylidene acetone	228 303 355	10,000 19,500 33,000	*trans*-β-Ionylidene ethylidene acetone	349	48,500*

Data of the UV absorption spectra of the thia-vitamin A series compared to those of the corresponding vitamin A series. The values indicated with* were measured in ethanol, the others in cyclohexane.

as well as on the inductive properties of the whole system of conjugated double bonds. The geometric position of the ring of the 4-thia-compounds in respect to the side chain is different from that of the corresponding ring in the vitamin A derivatives and the resonance system in the 4-thia-vitamin A series is extended by a lonely electronpair of the sulphur atom compared to the natural vitamin A analogues.

These factors in all probability must be responsible for the differenoes in the UV absorption spectra.

In the diagrams (1–4) and Table 1, the UV absorption spectra of the compounds of the 4-thia-vitamin A series and the corresponding ones of the vitamin A series are compared.

Biological data

Table 2 shows the results of the biological experiments:

TABLE 2

	Recovered part of the administered dose after 48 hr	
	All-trans-4-thia-vitamin A acetate	*All-trans* vitamin A acetate
Liver storage with chickens	8%	44%
Liver storage with rats	9%	46%

The growth potency test with chickens showed that *all-trans* 4-thia-vitamin A acetate was about 5% as active as the corresponding *all-trans* vitamin A acetate. Biological tests also indicated absence of toxicity at the dosis used (475 γ/kg feed) and absence of anti-vitamin A properties of 4-thia-vitamin A acetate.

EXPERIMENTAL

IR spectra were measured with an Unicam SP 200 spectrophotometer and the UV spectra with a Zeiss RPQ 20 C Spectrophotometer. (See Table 1 and diagrams 1–4.)

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 an internal reference. The spectrometer calibrations were checked by the procedure given by Jungnickel.[7]

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. For details of the NMR spectra we refer to part III[8] of this series.

trans-*Methyl 4-thia-ionylidene acetate* (II). Methyl diethylphosphonoacetate was prepared from triethyl phosphite and methyl bromoacetate.[8]

Methyl diethylphosphonoacetate (20·5 g; 0·1 mole) was added with stirring to a suspension of 2·3 g NaH in dimethoxyethane. To the clear solution 10 g (0·047 mole) 4-thia-ionone was added at room temp and the mixture refluxed for 4 hr. After extracting with pet. ether (b.p. 40–60°) and removing the solvent 12·25 g (98%) crude methyl 4-thia-ionylidene acetate was isolated. This consisted of a mixture of the *trans*- and the *cis*-isomers in a ratio of 75:25, as determined by NMR spectrometry.[3]

This mixture (10 g; 0·03 mole) was saponified with 10 g KOH in 50 ml water and 20 ml EtOH. After refluxing for 3½ hr and diluting with water the non acidic impurities were extracted with ether, the water layer acidified with dil. HCl and the acid extracted again with ether. After drying over $MgSO_4$ the solvent was removed *in vacuo* and 7·2 g (95%) crude acid isolated. By recrystallization from pet. ether (40–60°) 3 g of the *trans* 4-thia-ionylidene acetic acid was obtained in a pure state; m.p. 136–138·5°. (Found: C, 66·5; H, 7·9; S, 12·7. Calc. for $C_{14}H_{20}O_2S$ (252·37): C, 66·63; H, 7·99; S, 12·70%.)

The *trans* acid was converted into the corresponding *trans* methyl ester by diazomethane. IR spectrum (cap): C=O 1700 cm^{-1}; C=C 1600 cm^{-1}. NMR spectrum:[3] *trans* $C_{(9)}$-CH_3 δ = 2·32 (singlet); *trans* $C_{(8)}$-H δ = 6·07 (singlet).

trans 4-*Thia-ionylidene ethanol* (III). *trans*-Ionylidene methyl acetate (6·5 g; 0·024 mole) was reduced with 1·5 g LAH_4 in 150 ml ether at −15°. After 1 hr stirring at room temp the mixture was cooled to 0° and dil. H_2SO_4 added.

Upon ether extraction 6·5 g (100%) *trans* 4-thia-ionylidene ethanol could be isolated. IR spectrum (cap): C=C 1590 cm^{-1}; O—H 3375 cm^{-1}. NMR spectrum: $C_{(9)}$-CH_3 δ = 1·79, (singlet); $C_{(7)}$-H δ = 6·00 (singlet); $C_{(8)}$-H δ = 6·00 (singlet).

[7] J. C. Jungnickel, *Analyt. Chem.* **35**, 1985 (1963).

[8] G. M. Kosolapoff, *Organophosphorus Compounds* (1st Edition) Chap. 7. J. Wiley, N.Y. (1950).

trans 4-*Thia-ionylidene acetaldehyde* (IV). A mixture of 4·5 (0·018 mole) *trans* 4-thia-ionylidene ethanol, 50 g activated MnO_2 and 500 ml dry pet. ether (40–60°) were refluxed for 3 hr. The mixture was filtered and the solvent evaporated; 4·2 g (93%) crude *trans* 4-thia-ionylidene acetaldehyde were obtained.

The pure N,N-dimethylglycine hydrazone of the *trans* aldehyde was obtained by fractional crystallization according to the method described by Huisman;[9] m.p. 154–156·5°. (Found: C, 64·7; H, 8·9; S, 9·5; N, 12·5. Calc. for $C_{18}H_{29}N_3OS$ (335·54): C, 64·43; H, 8·71; S, 9·55; N, 12·52%.)

The pure *trans* aldehyde was regenerated from the hydrazone as described by Huisman,[9] yield 3·8 g. IR spectrum (cap): C=O 1660 cm^{-1}; C=C 1585–1615 cm^{-1}. NMR spectrum[8] of the *trans* aldehyde: *trans* $C_{(9)}$-CH_3, $\delta = 2{\cdot}29$.

trans *and* cis 4-*Thia-ionylidene ethylidene acetone* (V). *trans* 4-Thia-ionylidene acetaldehyde, (3·25 g; 0·013 mole) 65 ml 1N NaOH and 300 ml acetone were shaken in the dark at room temp for 16 hr. The mixture was poured into water, extracted with ether, washed with water and dried over $MgSO_4$. The solvent was evaporated and 3·85 g crude *trans* 4-thia-ionylidene ethylidene acetone was isolated. The crude ketone was converted into the N,N-dimethylglycine hydrazone according to Viscontini.[6]

After crystallization from ether the N,N-dimethylglycine hydrazone of 4-thia-ionylidene ethylidene acetone was obtained as a mixture of the *trans* and the *cis*-isomers, m.p. 110–114°. (Found: C, 67·4; H, 8·9; S, 8·4; N, 12·5. Calc. for $C_{21}H_{33}N_3OS$ (375·56): C, 76·15; H, 8·86; S, 8·54; N, 11·19%.) Fractional crystallization failed to isolate the pure *all-trans* isomer.

After regeneration with dil. HCl in EtOH, 1·8 g (90%) 4-thia-ionylidene ethylidene acetone was obtained from 2·8 g hydrazone. NMR spectrometry showed that the product consisted of a mixture of *trans* and *cis*-isomers in a ratio of ca. 70:30. IR spectrum (cap): C=O 1660 cm^{-1}; C=C 1570–1595 cm^{-1}.

All-trans 4-*thia-vitamin acid methyl ester* (VI). The above mixture of *trans* and *cis* 4-thia-ionylidene ethylidene acetones (4 g; 0·014 mole) were—with stirring—added to a clear solution of 6·3 g (0·03 mole) methyl diethylphosphonoacetate and 1·1 g NaH in 100 ml dimethoxyethane. After refluxing 4 hr, the mixture was stored for 14 hr at room temp in the dark and then poured into water and extracted with pet. ether (40–60°). After drying over $MgSO_4$, the solvent was evaporated and 4·5 g (98%) crude 4-thia-vitamin A acid methyl ester isolated. The crude ester was dissolved in 20 ml EtOH and added to 4 g KOH in 20 ml water. Then EtOH was added till a clear solution was obtained and the mixture refluxed for 4 hr. The mixture was poured into water and extracted with ether in order to remove non-acidic impurities.

The water layer was acidified with cold dil. H_2SO_4, extracted with ether and after drying over $MgSO_4$ and evaporating the solvent *in vacuo*, 4·05 g (95%) crude 4-thia-vitamin A acid were isolated. After fractional crystallization from MeOH the pure *all-trans* 4-thia-vitamin A acid could be obtained; m.p. 191–193°. (Found: C, 71·7; H, 8·2; S, 10·0. Calc. for $C_{19}H_{26}O_2S$ (318·46): C, 71·65; H, 8·23; S, 10·07%.)

The *all-trans* 4-thia-vitamin A acid was dissolved in ether and cooled to −40°. To this solution was added a solution of diazomethane in ether. After standing for 1 hr at −10° the ether and the excess of diazomethane were evaporated *in vacuo* and the *all-trans* 4-thia-vitamin A acid methyl ester isolated; (m.p. 63–34°). IR spectrum (cap): C=O 1700 cm^{-1}; C=C 1575–1615 cm^{-1}. NMR spectrum:[8] *trans* $C_{(13)}$-CH_3 $\delta = 2{\cdot}35$ (singlet); *trans* $C_{(9)}$-CH_3 $\delta = 1{\cdot}99$ (singlet).

All-trans 4-*thia-vitamin* A (VII). *All-trans* 4-thia-vitamin A acid methyl ester (400 mg) in 5 ml ether was added—with stirring—to a suspension of 70 mg LAH in 50 ml ether at −50°. After 2 hr more stirring at 10° the mixture was cooled to −50°, water added and the mixture acidified with cooled dil. H_2SO_4 at −5°.

After extraction with ether and drying over $MgSO_4$ the solvent was evaporated *in vacuo* and the *all-trans* 4-thia-vitamin A isolated; m.p. 36–37°.

The IR spectrum of this unstable compound was almost identical with that of vitamin A (Figs. 3 and 4). IR spectrum (cap): C=C 1575–1600 cm^{-1}; OH 3450 cm^{-1}.

All-trans 4-*thia-vitamin A acetate* (VIII). To a solution of 280 mg *all-trans* 4-thia-vitamin A in a mixture of benzene and 400 mg dry pyridine a solution of 300 mg acetyl chloride in 6 ml benzene was added with stirring. After 16 hr at 0° the mixture was diluted with water and extracted with pet. ether (40–60°).

[9] H. O. Huisman, A. Smit, P. H. van Leeuwen and J. H. van Rÿ, *Rec. Trav. Chim.* **75**, 977 (1956).

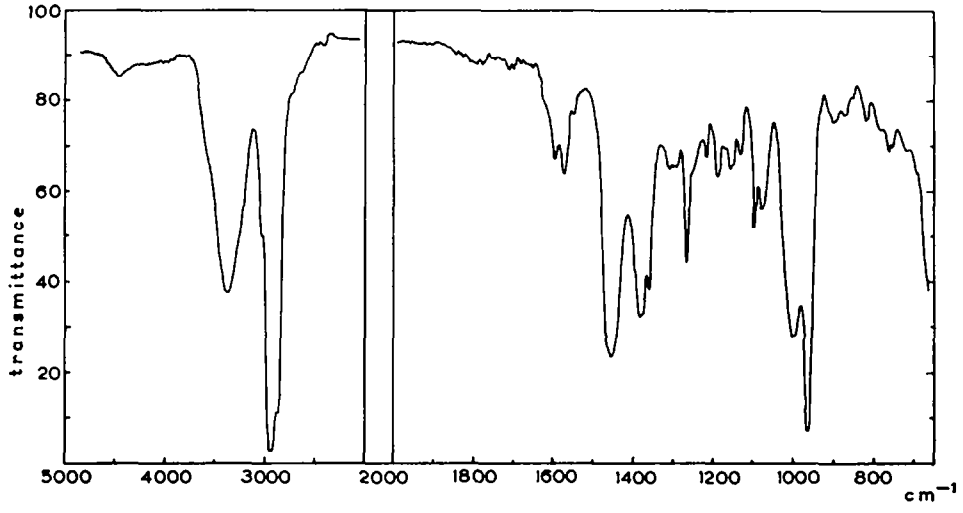


FIG. 3. *All-trans*-4-thia-vitamin A

The organic layer was washed with water, dil. H_2SO_4, $NaHCO_3$aq, again with water and dried over $MgSO_4$. After evaporation of the solvent, 311 mg *all-trans* 4-thia-vitamin A acetate was isolated. Attempts to obtain the acetate in a crystalline state failed. Only below zero is the substance crystalline. IR spectrum (cap): C=O 1740 cm^{-1}; C=C 1585–1600 cm^{-1}.

trans 4-*Thia-ionylidene cyanoacetic acid* (IX). A solution of 12 g (0·056 mole) 4-thia-ionone, 6·8 g (0·08 mole) cyanoacetic acid, 2·4 g ammonium acetate and 28 ml acetic acid in 60 ml benzene was refluxed for 3 hr. During the reaction the water formed was removed by means of a Dean Stark apparatus. The mixture was poured into ice water and after adding ether it was washed 3 times with a 5% NaOH solution.

The water layer was acidified with cold dil. HCl and the acid extracted with ether. After drying over $MgSO_4$ and removing the solvent 4 g (25%) 4-thia-ionylidene cyanoacetic acid could be isolated.

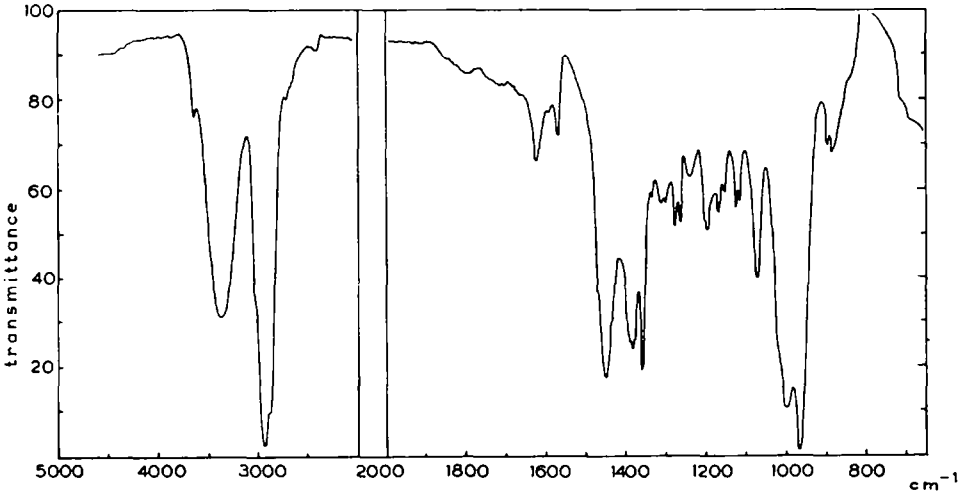


FIG. 4. *All-trans* vitamin A

After recrystallization from ether the *trans* isomer was obtained in a pure state; (m.p. 184–186°). (Found: C, 64·8; H, 6·9; N, 4·9; S, 11·6. Calc. for $C_{16}H_{19}NO_2S$ (227·37): C, 64·95; H, 6·90; N, 5·05; S, 11·56%.) IR spectrum ($CHCl_3$, 0·1 mm): C=O 1695 cm^{-1}; C≡N 2197 cm^{-1}, C=C 1500–1600 cm^{-1}.

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