TABLE II

SOME POLYTHIENYLS: ANALYTICAL DATA

Compound	Empirical formula	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Sulfur Calcd.	Sulfur Found	Mol. wt. Calcd.	Mol. wt. Found
α-Quaterthienyl	$C_{16}H_{10}S_4$	58.15	58.33	3.05	3.51	38.81	37.81	330	335
α-Terthienyl	$C_{12}H_8S_3$	58.03	57.97	3.25	3.54	38.73	37.70	248	242
2,2′-Bithienyl	$C_8H_6S_2$	57.79	57.99	3.64	4.05	38.57	37.90	166	163
1,10-Dimethyl-α-quaterthienyl	$C_{18}H_{14}S_4$	60.29	59.72	3.94	4.06	...	...	358	336
1-Methyl-α-terthienyl	$C_{13}H_{10}S_3$	59.50	59.22	3.84	3.94	36.66	35.62	262	259
1,8-Dimethyl-α-terthienyl	$C_{14}H_{12}S_3$	60.83	61.12	4.38	4.72	34.79	34.08	276	279
1,8-Dibromo-α-terthienyl	$C_{12}H_6S_3Br_2$	35.48	35.75	1.49	1.58	39.35	39.70	...	...

The analytical data are condensed in Table II. All samples, except bithienyl, were dried in high vacuum, at 56°, for two to four hours and stored *in vacuo*, in darkness. The sulfur was determined according to the Pregl "spiral tube" procedure for non-nitrogenous compounds.[5] Molecular weights were taken by the Rast method, in exaltone (except for quaterthienyl).

Summary

The chromatography of certain α-polythienyls is described and extinction curves are given.

PASADENA, CALIFORNIA RECEIVED AUGUST 10, 1946

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[5] H. Roth, "Quantitative Organic Microanalysis of Fritz Pregl," Blakiston, Philadelphia, 1937, p. 116.

[CONTRIBUTION NO. 1080 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

A Blue-fluorescing Compound, Terthienyl, Isolated from Marigolds

BY L. ZECHMEISTER AND J. W. SEASE

It was reported briefly, in collaboration with A. Sandoval,[1] that the yellow petals of the "Lemon" or "African" variety of the common marigold (*Tagetes erecta* L.) contain a substance which shows intense sky-blue or ink-blue fluorescence, both in solutions and in adsorbates. While the greenish-gray fluorescing phytofluene is very widespread in carotenoid-containing vegetable tissues,[1] the knowledge of the occurrence of the blue-fluorescing compound is so far restricted to the variety mentioned.

It was shown earlier that phytofluene is closely related to carotene; indeed, it is a colorless C_{40}-polyene hydrocarbon.[2] In contrast, the blue-fluorescing compound is spectroscopically quite different from the common carotenoids. Furthermore, it does not show any *cis–trans* isomerization[3] or an instantaneous coloration on acid earths.[4] The operations described below led to the isolation of crystals which in the crude state contained 32 to 35% sulfur but were free of oxygen or nitrogen. The analysis of a pure sample (38% sulfur) indicated a heterocyclic aromatic structure, in accordance with the strongly positive indophenine reaction. The sulfur cannot be removed from the molecule by heating with alkaline lead solution, which remains colorless. Likewise negative were tests for sulfhydryl groups. The whole behavior of the compound and the analytical data pointed to the class of the α-polythienyls, $C_4H_3S(C_4H_2S)_n$, C_4H_3S, and the molecular weight, which was found to be near 240, made a comparison with α-terthienyl (mol. wt., 248) advisable.

Since, in spite of Steinkopf's extended studies,[5] neither the quantitative separation of the members of the polythienyl series seemed to be entirely satisfactory for our purposes nor were spectral curves available, we recently made a limited experimental contribution to this field.[6] It was found that, under suitable conditions, polythienyls can be separated easily from each other on the alumina column. In contrast, α-terthienyl is not separable from our marigold compound under similar conditions. Both show a distinctly weaker adsorbability than higher members of the polythienyl series and 1-methyl- or 1,8-dimethyl-α-terthienyl. Furthermore, the natural product and synthetic α-terthienyl do not give any melting point depression.

The spectroscopic data obtained can be summarized as follows: In hexane solution ($\lambda_{max.}$ at 350 mμ) the compound isolated from marigolds

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[1] L. Zechmeister and A. Sandoval, *Arch. Biochem.*, **8**, 425 (1945).

[2] L. Zechmeister and A. Sandoval, THIS JOURNAL, **68**, 197 (1946); *cf.* H. H. Strain, *Nature*, **137**, 946 (1936); *J. Biol. Chem.*, **127**, 191 (1939).

[3] For a review of this field, *cf.* L. Zechmeister, *Chem. Rev.*, **34**, 267 (1944).

[4] The behavior of phytofluene was described by L. Zechmeister and A. Sandoval, *Science*, **101**, 585 (1945); the reaction itself was discovered by K. Takahashi and K. Kawakawi, *J. Chem. Soc. Japan*, **44**, 590 (1923).

[5] W. Steinkopf, "Die Chemie des Thiophens," Th. Steinkopff, Dresden, 1941; W. Steinkopf, R. Leitsmann and K. H. Hofmann, *Ann.*, **546**, 180 (1941).

[6] J. W. Sease and L. Zechmeister, THIS JOURNAL, **69**, 270 (1947).

shows an extinction curve which is distinguished from that of the carotenes or phytofluene by the absence of fine structure in its fundamental band. This is also valid for all synthetic polythienyls tested. Furthermore, the molecular extinction curves of the natural product and of synthetic α-terthienyl practically coincide, both in benzene and hexane solution. In the latter solvent, a second, much lower, peak was observed at 252 mμ (Fig. 1.). The position of the main maximum in Fig. 1 is different from those shown by methyl- or dimethyl-terthienyl.[6]

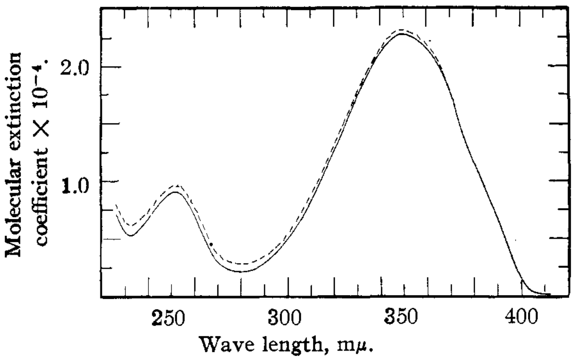

Fig. 1.—Molecular extinction curve in hexane of the blue-fluorescing compound as isolated from marigolds (full line). The dotted line refers to synthetic α-terthienyl sample and is given only where it does not coincide with the curve of the natural product.

From both the marigold compound and synthetic α-terthienyl the 1,8-dibromo derivative was prepared, according to Steinkopf, Leitsmann and Hofmann.[5] After chromatographic purification[6] the two samples were proved identical by mixed chromatogram and mixed melting point tests as well as by comparison of their molecular extinction curves. The respective crystal forms were also coinciding.

On the basis of all available evidence, the identity of the blue fluorescing compound from the petals with α-terthienyl, $C_{12}H_8S_3$, appears to be proved. However, the possibility cannot yet be entirely excluded that traces of isomeric terthienyls occur in the natural product, since no pertinent chromatographic or spectroscopic data are available. Perhaps such contaminants may be held responsible for a slight difference between the microscopic appearance of the natural and synthetic crystals.

So far as we know, the occurrence of terthienyl (or a similar substance) in plants has not yet been reported, and its role in the marigold remains problematical. Possibly, the absence of this sulfur-rich compound from other *Tagetes erecta* varieties, which were grown simultaneously in the same field, may attain some genetic significance.

Finally, it should be mentioned that natural α-terthienyl shows no provitamin A activity in the rat, and it is also void of antibiotic potency against microörganisms like *Staphylococcus aureus*, *Bac. subtilis*, *Escherichia coli* or *Pseudomonas ovalis*.

Acknowledgment.—Our thanks are due to Professor C. B. van Niel of the Hopkins Marine Station for antibiotic tests and to Prof. H. J. Deuel, Jr., of the University of Southern California for a vitamin A test. Furthermore, we are much indebted to Professor A. J. Haagen-Smit, Dr. G. Oppenheimer and Mr. G. Swinehart for micro-estimations.

Experimental

Materials and Methods.—The calcium hydroxide used was Shell Brand Lime, Chemical Hydrate, 98% through 325 mesh. A suitable alumina was found to be Alorco, Grade F, 80 mesh, reground to 200 mesh and mixed with 10% Celite 535. An adsorbent of similar strength was obtained by adding 0.7% water to ignited alumina.[7] Fluorescent zones were observed in the light of a "Purple X" incandescent ultraviolet lamp (General Electric Co.). Extinction curves were taken in a Beckman Spectrophotometer and represent averages of independent determinations. The melting points are corrected (Berl block; 2° per minute; substance under nitrogen).

Isolation.—A. Approximately 14.4 kg. of fresh petals, corresponding to 10,000 flowers, were collected in Southern California in December. The material was covered with methanol and ground to a slurry in a Waring blendor within twenty-four hours after picking. Separation in a basket centrifuge (diameter, 20 cm.) yielded 40 liters of extract.[8] The latter, in portions, was concentrated *in vacuo* to 2.5 liter. Solids which precipitated during this process were, after decantation, dissolved in petroleum ether (b.p. 60–70°). When kept at 4° both the methanolic and the petroleum ether solutions deposited new precipitates, each of which was filtered off and washed with petroleum ether until the flow no longer fluoresced in ultraviolet light.

The methanolic solution was discarded, and the petroleum ether solution was combined with all washings (total, 1.0 liter).

In order to remove a fine suspension which would have clogged columns, the solution was sucked through a 2-cm. layer of calcium carbonate (Merck, Heavy Powder), and the solid was washed free of fluorescent material with petroleum ether. The combined filtrates (about 1.7 liter) were adsorbed on four calcium hydroxide columns (30 × 8 cm.), which were then washed with petroleum ether until the percolate no longer fluoresced. Several colored zones remained adsorbed. The combined four chromatographic filtrates (4 liter) were evaporated *in vacuo* to 200 ml. and developed with petroleum ether on a 24 × 8 cm. alumina column (the figures on the left denote the thickness of the zones in mm.; fl. = fluorescence or fluorescent).

5 colorless, includes faint blue-fl. streaks
2 orange, no fl.
5 pink, no fl.
5 interzone
15 pale yellow, faint bluish fl.
35 colorless, strong blue fl.
13 colorless, blue fl., different from the 35 mm. zone[9]
160 empty bottom zone

The 35 mm. zone was eluted with ether and evaporated

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[7] P. B. Müller, *Helv. Chim. Acta*, **26**, 1945 (1943); **27**, 404 (1944).

[8] Three subsequent re-extractions of the petals with a biphasic methanol + petroleum ether mixture gave a solution in which an additional amount of blue-fluorescing compound was found photometrically ($^1/_4$ of that contained in the methanol). We were unable to isolate crystals from this fraction.

[9] This zone contained an uninvestigated compound whose spectral curve is similar in shape to that of terthienyl, but shows an extinction maximum at 341 mμ in petroleum ether.

to give a mixture of crystals and a non-volatile oil. The total was dissolved in 0.75 ml. of hot petroleum ether. Upon cooling to 0° only crystals appeared. Recrystallization from 3 ml. of petroleum ether gave 198 mg. of crude material, m. p. 81–84°. The mother liquors yielded an additional 16 mg. The total corresponds to 15 mg. per kg. of fresh marigold petals.

B. In a second experiment 9.5 kg. of petals was extracted three times with cold methanol. Each of these solutions was concentrated and chromatographed from petroleum ether, first on calcium hydroxide and then on alumina as described above. The blue fluorescing main zone obtained on alumina from the first extract was chromatographically homogeneous, while those from the second and third extracts were contaminated with phytofluene (about 1 mg. and 4 mg., respectively), which could not be eliminated chromatographically. Therefore, the phytofluene-containing eluates were transferred into petroleum ether and shaken ten times with 90% methanol which had been previously saturated with petroleum ether. Phytofluene remained in the epiphase but the blue-fluorescing compound migrated into the hypophase from which it was transferred into petroleum ether. Combination of all hypophasic material with that originating from the first methanolic extract gave 4 ml. of an oil on evaporation.

The latter was dissolved in 250 ml. of petroleum ether and saponified by standing over 15–20 ml. of 20% methanolic potassium hydroxide for two days during which period the alkali was renewed four times. Finally, the combined alkaline layers were diluted with 1 vol. of water and extracted ten times with petroleum ether, the extracts being added to the main petroleum ether solution. After thorough washing with water, drying over sodium sulfate and evaporation to 50 ml., the solution was developed with petroleum ether on a 25 × 8 cm. alumina column. The main fluorescing zone was eluted with ether and evaporated to give a mixture of crystals and non-volatile oil (total, 1 ml.) which was dissolved in 1.5 ml. of light petroleum ether (b. p. 28–38°). Upon cooling to 0° crystals appeared. Three recrystallizations from methanol yielded 125 mg.; m. p. 91–93.5°. The mother liquors gave 75 mg. The total yield corresponds to 21 mg. per kg. of fresh petals.

Analytical Data.—Samples were stored *in vacuo* and darkness. All parallel determinations were carried out with independently prepared samples.

Anal. Calcd. for $C_{12}H_8S_3$: C, 58.03; H, 3.25; S, 38.73. Found: C, 58.18, 58.17, 58.41; H, 3.41, 3.50, 3.42; S, 37.96. The two first samples were ash-free; the third result (C and H) has been corrected for 0.9% ash.[10]

Molecular Weight.—(*a*) Rast micro method: 0.272 mg. of substance in 2.027 mg. of exaltone (k = 21.3): Δ = 12.4°; 0.422 mg. in 3.397 mg.: Δ = 11.1°; 0.412 mg. in 5.218 mg.: Δ = 6.9°; 0.292 mg. in 2.489 mg.: Δ = 10.6°. (*b*) Cryoscopic macro method: 35.86 mg. of substance in 6.121 g. of benzene (k = 5.1): Δ = 0.122°. Calcd. for $C_{12}H_8S_3$: mol. wt., 248. Found: (*a*) 231, 238, 244, 236, average, 237; and (*b*) 245.

Dibromo Derivative.—Calcd. for $C_{12}H_6S_3Br_2$: C, 35.48; H, 1.49; Br, 39.35. Found: C, 35.70; H, 1.66; Br, 39.42.

Properties.—Under the microscope the compound shows either long prisms with dented ends or quadrangular plates which tend to become irregular and form complicated groups. The macroscopic color is lemon yellow, which may darken upon exposure to air and light. The melting point of the best samples was 94–95.5° and was not depressed by synthetic α-terthienyl. The compound is very easily soluble in carbon bisulfide, ether, benzene, acetone but somewhat less in petroleum ether. The solubility in methanol or ethanol is still considerable; the former solvent is suited for crystallizations. Upon partition between petroleum ether and 75 to 95% methanol, the greater part of the compound is found in the epiphase. The solutions may be stored unaltered in darkness at 4°, under carbon dioxide, for several weeks. All solutions showed an intense blue fluoresence in ultraviolet light. The benzene solution was found to be optically inactive. The extinction curve in hexane is represented in Fig. 1. A practical identity of the extinction values with those of synthetic α-terthienyl was also found in benzene solution.

The compound is easily adsorbed from petroleum ether on alumina of sufficient strength but is washed rapidly through calcium hydroxide by the same solvent. The chromatograms on alumina are best developed with petroleum ether containing 1 to 3% acetone, depending on the strength of the adsorbent. A mixture of benzene and petroleum ether (1:10) can also be used. Because of overlapping, a clear separation from phytofluene was not obtainable. Thus, in the chromatogram of some extracts the broad, blue fluorescing zone contained a thin streak of the more greenish fluorescing phytofluene upon development with pure petroleum ether. Mixed chromatogram tests on alumina with quaterthienyl, 1-methyl-terthienyl or 1,8-dimethyl-terthienyl showed clear separations when developed with benzene + petroleum ether (1:10); but petroleum ether and 1 to 3% acetone is unsuitable for separating terthienyl from its methyl derivatives. In no case did a separation of the marigold compound and synthetic α-terthienyl occur.

Concentrated sulfuric acid gives with either the natural product or synthetic α-terthienyl a permanganate-like solution; the corresponding color obtained with quaterthienyl is purplish, while that with bithienyl is an uncharacteristic brown-yellow. If the substance is added to a yellow solution of isatin in concd. sulfuric acid, both the natural product and synthetic terthienyl first develop the permanganate-like color mentioned, which, however, in the course of a few minutes, changes to deep indigo blue (indophenine reaction). Bithienyl or quaterthienyl go through the first phase much more rapidly and show a deep greenish-blue or blue coloration almost immediately. Crystals of trichloroacetic acid in contact with those of natural or synthetic terthienyl develop a red coloration which later becomes greenish.

Summary

The petals of the "Lemon" ("African") variety of the common marigold (*Tagetes erecta* L.) contain a blue-fluorescing compound, α-terthienyl, $C_{12}H_8S_3$, which has been isolated in pure crystals.

PASADENA, CALIFORNIA RECEIVED AUGUST 10, 1946

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(10) For less pure samples the sulfur content was lower by 1 to 3% which involved a corresponding increase of C + H. In such a case the substance included a crystallizable contaminant with a much higher C and H content than our best product.