Occurrence of a Plastochromanol in Linseed Oil

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In connection with studies on production of encephalomalacia in chicks with diets containing vegetable oils [1] we analyzed samples of vegetable oils for individual tocopherols, using column and thin layer chromatography. Besides α-, β-, γ-, and δ-tocopherols we found an unidentified substance X occurring in that region of the thin layer chromatogram, where the dimethyl-tocotrienols are expected, but not where β-tocotrienol (5,8-dimethyl-tocotrienol) is found. The substance occurred in soybean oil, rapeseed oil, and cottonseed oil and, most abundantly, in linseed oil where it constituted approximately 29 % or 200 μg of the total tocopherols, the amount of which was 690 μg/g oil. It seemed possible that substance X could be γ-tocotrienol (7,8-dimethyl-tocotrienol, plastochromanol-3), but we had no standard representing the latter compound.

Through the courtesy of Dr. J. F. Pennock, Department of Biochemistry, University of Liverpool, we have recently received samples of γ-tocotrienol (γ-T-3, PC-3) and plastochromanol-8 (PC-8). The latter substance has been found to occur in leaves of *Hevea brasiliensis* and identified with the synthetically produced compound of the same designation. PC-8 has also been found in rapeseed oil and in maize oil.[2]

Using these two substances as standards we have found that our substance X from linseed oil is different from γ-T-3 and behaves like PC-8.

By two-dimensional thin layer chromatography according to Whittle and Pennock,[3] a mixture of γ-tocopherol (γ-T), substance X from 0.1 g linseed oil, and 15 μg PC-8 gave only two spots, whereas γ-T, substance X from 0.1 g linseed oil, and γ-T-3 gave three spots.

In gas-liquid chromatography of the trimethylsilyl ethers of γ-T-3, substance X, PC-8, and α-tocopherol on butandiol succinate 4 % (column temp. 220°C), γ-T-3 gave a peak with a retention time relative to α-tocopherol of 1.42 and a smaller peak with a relative retention time of 0.35, whereas under the same conditions substance X and PC-8 gave no peaks. This confirms that substance X cannot be γ-T-3.

The ultraviolet spectra in cyclohexane showed absorption maxima at 294 and 300 nm for substance X, PC-8, and γ-T-3.

In absolute ethanol, the three substances showed a maximum covering the wavelengths 294 to 300 nm. In ethanol, $E^{1\,\%}_{1\,cm}$ at 296 nm was 55.5 for PC-8 and 68.5 for γ-T-3.

The ratio between E_{296} and E_{520} was 0.184 for substance X, 0.175 for PC-8, and 0.178 for γ-T-3. (E_{520} refers to the intensity of the color in the Emmerie-Engel reaction carried out as described by Bro-Rasmussen and Hjarde [4]).

The nitroso derivatives of substance X, PC-8, and γ-T-3 were prepared by the method of Marcinkiewicz and Green.[5] In petroleum ether the nitroso derivatives of the three substances showed an absorption minimum at 358 nm and an absorption maximum at 416 nm. By thin layer chromatography on silica gel GF (254, 360) Merck with di-isopropylether:petroleum ether (4:96) as mobile phase, the nitroso derivatives of substance X and PC-8 moved with the same R_F (0.59), whereas the nitroso derivative of γ-T-3 moved slower (R_F = 0.53).

Thus, in all the tests used, substance X behaves like plastochromanol-8 (PC-8).

One of us (E.L.) has previously found substance X, now identified with plastochromanol-8, in eggs from hens fed linseed.

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