uniformity of mayonnaise as a quality control method by measuring ethyl acetate and acetic acid for vinegar content and allyl isothiocyanate for mustard content in mayonnaises.

This report is mainly concerned with the development of an analytical method which can measure the changes of flavor compounds of mayonnaise during storage periods. It will be necessary to analyze numerous mayonnaises with a wide range of sensory scores to determine how well the instrumental gas chromatographic analyses can be correlated with sensory scores.

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[Received November 16, 1981]

Functionalization at the Double Bond Region of Jojoba Oil: II. Diels-Alder Adducts of Jojobatetraene

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ABSTRACT

Diels-Alder products derived from jojobatetraene and several dienophiles are synthesized and described. Singlet oxygen forms a cyclic peroxide. The adducts introduce a new line of chemicals derived from jojoba oil.

INTRODUCTION

Increasing unsaturation in the form of conjugated double bonds along the chain of jojoba oil can serve several purposes such as enhancing oxidation capability, and enabling the chemist to widen and diversify a host of chemical reactions, as a result of increased polarizability of the π electrons. We report here on the chemical reactivity of jojobatetraene (III), which is readily available from jojoba oil (I) by NBS bromination and subsequent HBr elimination (1) (see scheme in Fig. 1). We report on the Diels-Alder reaction of several dienophiles whose products introduce a new line of chemicals derived from jojoba oil.

EXPERIMENTAL PROCEDURES

General

The crude product after each chemical transformation was used without further purification for the next step. The usual work-up consisted of pouring the reaction mixture into H_2O, extraction with petroleum ether (60-80), washing with saturated NaCl solution, and drying over anhyd Na_2SO_4. Infrared (IR) and nuclear magnetic resonance (NMR) spectra provided monitors for the chemical change occurring in each reaction. Purity was determined by NMR (2,3). All NMR spectra gave the following: terminal CH_3 at δ 0.92-0.94; an intense signal at 1.2-1.4 for all aliphatic hydrogens; a signal at 1.98-2.05 for allylic hydrogens. Other signals are mentioned later. Integration curves were consistent with the assignment of the different hydrogens. The NMR spectra were determined on a Varian XL-100 in CCl_4 or $CDCl_3$ solution. The IR spectra were determined with a Perkin Elmer 377, usually neat or in $CHCl_3$ solution.

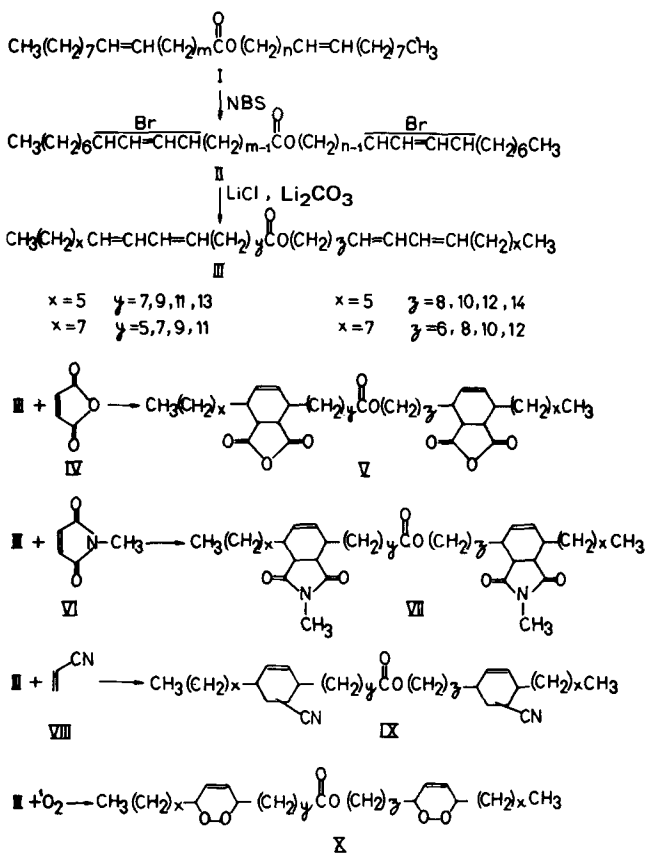


FIG. 1.

Analytical thin layer chromatography (TLC) plates (20 x 20 or 20 x 10 cm and 0.1 mm thickness) were prepared with Silica Gel KGS-254. Preparative thick layer chromatography (PLC) plates (20 x 20 cm and 1 mm thickness)

were prepared with silica gel. Crude samples of 70-90 mg were loaded on each of the PLC plates for purification. Eluting system was 10% of ether in petroleum ether (60-80) with recovery of 70-80%, by extracting with ether.

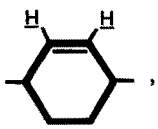
SOLVENTS

Petroleum ether (60-80) was dried over $CaCl_2$ and distilled. Ether was dried over $CaCl_2$, then over Na and distilled. Toluene was dried over Na. Ethanol and *t*-BuOH were chemical pure and used without drying or purification.

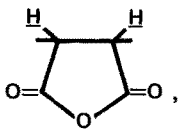
Jojobatetraene (III) was 75-80% pure and contained 65-70% of (*E,E*)-isomer and 30-35% of (*Z,E*)-isomer (1). No exhaustive purification of the products was tried, but the products (Fig. 1) could be identified through their NMR spectra.

Bismaleic Anhydride Adduct (V)

A solution of 0.38 g of jojobatetraene (III) (0.6 mmol) and 0.15 g maleic anhydride (IV) (1.4 mmol) in 5 mL toluene was refluxed for 10 hr to yield 0.5 g of crude V. IR 1690-1760 cm^{-1}. NMR 5.70 (2H,



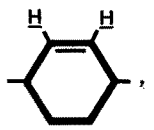
bs), 5.20-6.10 (2H, -CH=CH-, m), 3.96 (2H, $-CH_2OCO$, t, J=7 cps), 3.30 (2H,



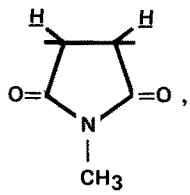
bs), 2.10-2.40 (4H, $-CH_2COO$, m). A sample of 0.2 g was purified on PLC (elution with 10% ether in petroleum ether) to yield 20 mg of unreacted (*Z,E*)tetraene, and 100 mg of V in 80% purity. NMR 5.70 (3.2H), 3.96 (2H), 3.30 (3.2H).

Bis N-methylmaleimide Adduct (VII)

A solution of 0.2 g III (0.33 mmol) and 78 mg of maleimide (VI) (0.7 mmol) in 5 mL ethanol was refluxed for 5 hr to yield 0.25 g of crude VII (~50% purity). IR 1680-1740 cm^{-1}. NMR 5.70 (2H,



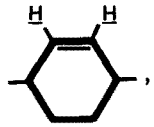
s), 5.20-6.10 (2H, -CH=CH-, m), 3.96 (2H, $-CH_2OCO$, t, J=7 cps), 3.50 (2H,



bs), 2.80 (3H, N-CH_3, s), 2.10-2.40 (5H, $-CH_2COO$, m).

Bisacrylonitrile Adduct (IX)

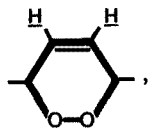
A mixture of 0.3 g III (0.5 mmol) and 2 mL of acrylonitrile (VIII) (large excess) in a stainless steel reactor (3) was heated at 120 C for 48 hr to yield 0.22 g of crude IX. Purification on PLC yielded 0.12 g of IX (80% purity). IR 2260, 1710 cm^{-1}. NMR 5.60 (3.2H,



bs), 5.20-5.80 (1.6H, -CH=CH-, m), 3.96 (2H, $-CH_2OCO$, t, J=7 cps), 2.70-2.90 (1.6H, CH_2-CHCN, m), 2.22 (2H, $-CH_2COO$, t, J=7 cps). Calcd for N, 3.94%; found, 3.2%.

Biscyclic Peroxide (X)

A solution of 0.4 g III (0.66 mmol) and 40 mg of Rose bengal in 15 mL *t*-BuOH was left in sunlight for 10 hr, after which the solvent was evaporated to leave 0.4 g of viscous liquid. IR 3350-3450, 1710, 1180 cm^{-1}. NMR 5.70 (0.2H,



s), 5.20-6.10 (1.8H, -CH=CH-, m), 4.10-4.30 (1.5H,

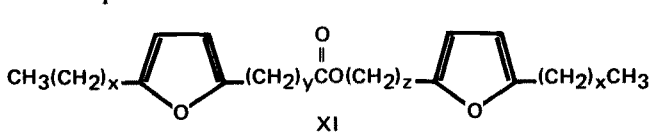
and -CH=CHCHOH, m), 3.94 (2H, $-CH_2OCO$, t, J=7 cps), 2.20 (2H, $-CH_2COO$, t, J=7 cps).

RESULTS AND DISCUSSION

Three typical dienophiles have been selected for the chemical transformation: maleic anhydride (IV), N-methylmaleimide (VI) and acrylonitrile (VIII). The fourth one is singlet oxygen ($^1O_2$), which might shed some light on oxidation processes in diunsaturated fatty acids and drying oils.

The reactivity of the tetraene with the maleic acid derivatives (IV,VI) is somewhat lower than short-chain conjugated dienes which are usually reactive enough to complete the reaction rapidly and under mild conditions. In our case, only after several hr of reflux were the products formed. In the case of acrylonitrile, the reactivity was found to be even lower, and only much more drastic conditions (sealed reactor) could force the formation of the adduct.

It was interesting to study the singlet oxygen reaction, which typically reacts with conjugated dienes (4, and R. Idesis et al., unpublished data) to form the cyclic peroxide X. This product can lead to a furan derivative such as XI:



XI

x=5, y=7,9,11,13 x=5, z=8,10,12,14
x=7, y=5,7,9,11 x=7, z=6,8,10,12

Furano fatty acids are known as natural products (5).

Excess of dienophiles were taken for the reaction and no monoadducts were isolated. As the tetraene III was not pure, the products always contained up to 30-35% of the starting (*Z,E*)-diene unit on each side of the tetraene. This group usually does not react to yield Diels-Alder products. This fact explains the relative lower yield of the bisadducts.

Introduction of the reactive functional groups via Diels-Alder reaction opens a new line of chemicals which can be derived from these intermediates of jojoba oil.

ACKNOWLEDGMENT

Dalia Gold provided technical assistance.

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[Received August 26, 1981]

⁂Effect of Temperature on Soybean Seed Constituents: Oil, Protein, Moisture, Fatty Acids, Amino Acids and Sugars

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ABSTRACT

Soybean plants were grown at day/night temperatures of 24/19 C until the beginning of seed development, and then transferred to 5 different temperature regimes (18/13, 24/19, 27/22, 30/25 and 33/28 C) in the CSIRO phytotron. Mature seeds that developed under these conditions were analyzed for variances in composition. Fatty acid composition was strongly affected by temperature: linolenic and linoleic acids decreased markedly whereas oleic acid increased as the temperature increased; palmitic and stearic acids remained unchanged. Oil content was positively correlated with temperature, and protein content increased at the highest temperature. Of the sugars analyzed, sucrose concentration decreased by 56% with a 15 C increase in temperature, and stachyose showed a slight reduction; other sugars remained unchanged. Amino acid composition was generally stable; however, methionine increased with increased temperature during seed development. Moisture content was unaffected.

INTRODUCTION

It is generally thought that oilseed plants grown under a warmer climate produce seeds containing less highly unsaturated fat than when grown under a colder environment. For example, Collins and Howell (1) found in soybeans that linoleic and, more strongly, linolenic acids are negatively correlated with temperature, using Weather Bureau data to substantiate the difference in growing temperatures of the locations studied. Howell and Collins (2), using both location and greenhouse studies, confirmed these results. Collins and Sedgwick (3) found that soybean varieties grown at the northern end of their range produced oil 1-2 percentage points higher in linolenic acid and 3-6 higher in linoleic acid than when grown at the southern end of their range. More recently, Chapman et al. (4) showed that soybeans grown at Tifton, Georgia (with an average daily temperature of 25.7 C), have 2% lower linoleic and 20% lower linolenic acid content, with 11% higher oleic, than the same varieties grown at Blairsville, Georgia (with an average daily temperature of 18.0 C).

Temperature has also been shown to affect total oil and protein content of soybeans. Howell and Cartter (5,6) reported a positive correlation between maximal temperature and oil percentage. Additionally, both a negative (7-9) and a positive (4) correlation between protein and oil have been reported. Howell and Cartter (6) found that protein was stable with relation to temperature, but non-protein nitrogen increased as the temperature rose.

Little work has been done on the other constituents of the soybean seed as they relate to temperature during seed growth. Chapman et al. (4) found that moisture content decreased as growing temperature increased. Hymowitz et al. (9) reported that total sugar content of the soybean seed, as well as sucrose alone, is positively related to total oil and negatively to total protein. However, no correlation was made between this component and temperature, nor has the relationship between amino acids and temperature been explored.

None of the studies discussed above were conducted under completely controlled environmental conditions. The present experiment undertook this task, using soybeans of one variety (Fiskeby V) that were grown in a phytotron under 5 different controlled day/night temperature regimes during seed maturation. All the major constituents of soybeans were analyzed: oil and fatty acids, protein, sugars, amino acids and moisture.

EXPERIMENTAL PROCEDURES

Dr. D.B. Egli kindly supplied soybeans, variety Fiskeby V, which had been grown in the CSIRO phytotron, Australia, at day/night temperatures of 24/19 C (8-hr day) until the beginning of seed growth, and then shifted to the following day/night temperature regimes until maturity: 18/13 C; 24/19 C; 27/22 C; 30/25 C; and 33/28 C (10). All analyses of seed constituents were run in duplicate.

Oil content was determined by Butt Extraction (11) with petroleum ether as solvent. Fatty acids were esterified according to the method of Metcalfe et al. (12), and analyzed on a Varian 3700 gas chromatograph using a 6 ft x 2 mm glass column packed with 5% LAC-2-R 446 on Gas-Chrom Q at 180 C.

Amino acid composition was determined by hydrolyzing a known amount of ground, defatted sample and an internal standard (Norleucine) in 6 N HCl and analyzing on a Glenco MM-100 amino acid analyzer. Average nitrogen recovery was 95%. Methionine and cystine were determined according to the method of Moore (13).