Photolysis of Nootkatone

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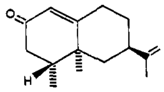
The photolytic decomposition of nootkatone, the principal flavoring constituent in grapefruit, yields a variety of products. The major component, photonootkatone, has been isolated and shown to have structure VIII.

Since the pioneering work of Ciamician and Silber (1908) and Paterno and Chieffi (1909) on the light-induced transformation of organic compounds, tremendous strides have been made not only in the elucidation of photochemical mechanisms but also in utilization of photolysis as a synthetic "tool." The application of photochemistry within the past few years by the organic chemist has resulted largely because of progress in analytical techniques, methods of separation, etc.

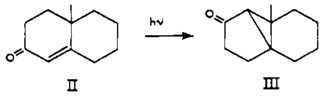
In the food industry and allied fields, a tremendous effort has been put forth to elucidate the mechanism of photoreactions which often produce off-flavors or other undesirable qualities. Considerable effort has been exerted to determine the exact mechanism of photo-induced rancidity, especially in the area of fats and oils (Coe, 1938, 1939, 1941). Outside of this particular area, little definitive work has been done on the changes which occur when flavoring constituents of beverages, fruits, etc., are exposed to ultraviolet light.

The principal flavoring compound of grapefruit (MacLeod, 1964), nootkatone, was shown by MacLeod in 1965 to have structure I. Since that time, considerable interest has been shown by the food industry in the utilization of this sesquiterpene ketone in various food products, especially soft drinks. Although MacLeod (1964) showed that nootkatone is relatively stable to heat and basic and acidic conditions, its stability upon irradiation with ultraviolet light was largely unknown.



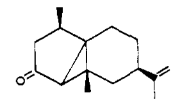
I

The photochemical investigation of α, β-unsaturated cyclic ketones has received considerable attention (Chapman, 1967), and one might, *a priori*, expect nootkatone to react in a similar manner—for example, the ketone (II) upon irradiation gives the transformation product (III) (Zimmerman *et al.*, 1966).



II III

The corresponding product from nootkatone would be IV.



IV

One other interesting feature of nootkatone is that it bears, in addition to the conjugated double bond, an isopropenyl group which one might expect to give an intramolecular cycloaddition product analogous to the conversion of carvone (V) to carvonecamphor (VI), as was shown by Paterno and Chieffi (1909) and Büchi and Goldman (1957). The activated double bond of nootkatone is, however, one carbon further removed from the nonactivated one. It then becomes of additional interest to determine if this intramolecular reaction might indeed occur.

V VI

EXPERIMENTAL

Nuclear Magnetic Resonance Spectra. All spectra were run in dilute carbon tetrachloride solutions using tetramethylsilane as an internal standard. A Varian HR 100 spectrometer was used.

Infrared Spectra. A Perkin-Elmer 237 infrared spectrometer was used, in which the samples were either run neat or in carbon tetrachloride.

Raman Spectra. A Perkin-Elmer LR-1 Raman spectrometer was used and all samples were run neat using a microtechnique (Bailey *et al.*, 1967). Solid samples were heated until they melted.

Mass Spectra. A Bendix Model 12 Time-of-Flight mass spectrometer and a CEC Model 110 high resolution mass spectrometer were used.

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Nootkatone. Fivefold grapefruit oil was distilled at 23°C. and 10-micron pressure to remove low boiling constituents. The temperature was then raised to 55°C. and an intermediate fraction distilled off. The residue remaining was then distilled through a falling-film molecular still in which the pressure was held at 1 micron and the temperature of the outer jacket kept at 70°C. The distillate was cooled, and crystalline nootkatone separated and was filtered and washed with heptane. Gas chromatographic analysis on a 75-foot × 0.01-inch Igepal column showed the material to be about 98% nootkatone.

Photolysis of Nootkatone. To a 1-liter flask fitted with a nitrogen bubbler, reflux condenser, and Hanovia immersion-type photocell were added 1 gram (4.6 mmoles) of nootkatone and approximately 250 ml. of anhydrous methanol. A 450-watt high pressure mercury lamp was used, with a borosilicate glass filter to irradiate the solution. After 24 hours, the lamp was removed and the solution diluted with 1 liter of water. The cloudy mixture (some solid material was present) was then extracted with heptane, which was washed with water and dried over anhydrous magnesium sulfate. After filtering and evaporation of the solvent, 0.75 gram of a light yellow oil remained. The oil was then chromatographed on a column of silica gel (100- to 200-mesh) using heptane-ether (80 to 20, v./v.) as the eluent. The first fraction to be eluted was photonootkatone (168 mg., 14.5%). Gas chromatographic analysis on a 75-foot × 0.01-inch Igepal column showed the material to be homogeneous.

Irradiation of nootkatone with oxygen bubbling through the solution completely suppressed the reaction.

Norphotonootkatone. Photonootkatone (250 mg., 1 mmole) was dissolved in 15 ml. of purified tetrahydrofuran and 2 ml. of pyridine. To this solution was added 500 mg. (1.97 mmoles) of osmium tetroxide with 5 ml. of tetrahydrofuran. The solution was stirred at room temperature for 15 minutes, after which time it became dark; then 914 mg. (4 mmoles) of periodic acid was added along with 15 ml. of water. The mixture was stirred at room temperature for 19 hours, then cooled to 0°C. Sodium sulfite (3 grams) dissolved in 20 ml. of water was then added and the mixture stirred for 30 minutes, after which time it was allowed to warm to room temperature and was stirred for an additional 30 minutes. The solid material was filtered off and the filtrate diluted with a large amount of water. The mixture was then extracted with ether and the ethereal extract washed with water, 10% sodium thiosulfate solution, dilute hydrochloric acid, dilute sodium hydroxide, and finally water. After drying over anhydrous magnesium sulfate, the ether was removed, leaving a dark oil which was chromatographed on a column of silica gel (100- to 200-mesh). Heptane-ether (80 to 20, v./v.) was used as the eluent. After photonootkatone was eluted, and another small fraction which showed hydroxyl absorption in the infrared, norphotonootkatone (171 mg., 68%) was eluted.

Nootkatone-d_5. Nootkatone (200 mg.) was dissolved in 5 ml. of purified dioxane and to this solution were added 0.5 ml. of deuterium oxide and 10 mg. of anhydrous potassium carbonate. The mixture was refluxed for 10 minutes, then evaporated to dryness. The process was repeated four times, whereupon the solution was diluted with ether and dried over anhydrous magnesium sulfate. After filtration and evaporation of the solvent a light yellow oil remained which by NMR analysis showed approximately 88% deuterium at each active site. The photolysis of nootkatone-d_5 was carried out in the usual manner.

RESULTS AND DISCUSSION

Gas chromatographic analysis of the crude photolyzed nootkatone, shown in Figure 1, indicates that nootkatone is reactive and subject to modification by the action of ultraviolet light. Purified photonootkatone (first major peak) is a high boiling liquid with a rather bland nondescript odor and shows infrared absorption peaks (Figure 2) at 1743 and 1165 cm.$^{-1}$ (aliphatic ester); 3078, 1622, and 887 cm.$^{-1}$ ($>C = CH_2$); 1387 and 1370 cm.$^{-1}$ (*gem*-dimethyl).

The mass spectrum of photonootkatone (Figure 3) shows a parent peak at *m/e* 250 with additional peaks at *m/e* 235 (M-15) and *m/e* 219 (M-41). High resolution mass spectral analysis gave *m/e* 250.1943; calculated for $C_{16}H_{26}O_2$, 250.1932. Methyl ester and terminal methylene groups are readily confirmed by the NMR spectrum of photonootkatone (Figure 4). A singlet (3 *H*) at 359 c.p.s. is typical of aliphatic methyl esters; a poorly resolved doublet (2 *H*) at 480 c.p.s. is also typical of a terminal methylene group. Two singlets (3 *H* each) at 81 and 85 c.p.s., which are not spin-spin coupled as indicated by comparing the spectrum with one run at 60 Mc., can be assigned to the *gem*-dimethyl group in which each methyl moiety is in a different environment. In addition a singlet (3 *H*) at 124 c.p.s. indicates the presence of a tertiary allyl methyl group. A poorly resolved doublet (3 *H*) at 177 c.p.s. can be ascribed to a vinyl methyl group. The small splitting of this peak is reflected in the splitting of the vinyl protons (480 c.p.s.).

A pair of related doublets (1 *H* for each doublet) are found with centers at 272 and 217 c.p.s. $J = 16$ c.p.s. The rather large coupling constant is indicative of *gem* coupling of a methylene group. The chemical shift also indicates that the methylene group is attached to the ester moiety. The peaks are not further split, indicating the methylene to be attached to a tertiary center. The assignment of the pair of doublets was confirmed by reducing photonootkatone with lithium aluminum hydride in which the ester group was reduced to a primary alcohol. The NMR spectrum showed the expected upfield shift of the peaks along with the loss of the methyl singlet associated with the ester.

Oxidation of photonootkatone with osmium tetroxide–sodium periodate gave norphotonootkatone again, an oil with a molecular weight of 252 (by mass spectrometry, Figure 5). The infrared spectrum no longer showed the characteristic peaks for a double bond but showed an additional intense peak at 1700 cm.$^{-1}$ ($>C = 0$). The NMR spectrum (Figure 6) confirms the absence of vinyl protons but still shows a methyl ester (357 c.p.s., 3 *H*), a deshielded singlet (127 c.p.s., 3 *H*), and two high-field singlets (3 *H* each) at 86 and 67 c.p.s., one of which has shifted upfield relative to photonootkatone. Norphotonootkatone still shows the pair of doublets assigned to a methylene adjacent to the ester group. The poorly resolved doublet (177 c.p.s.,

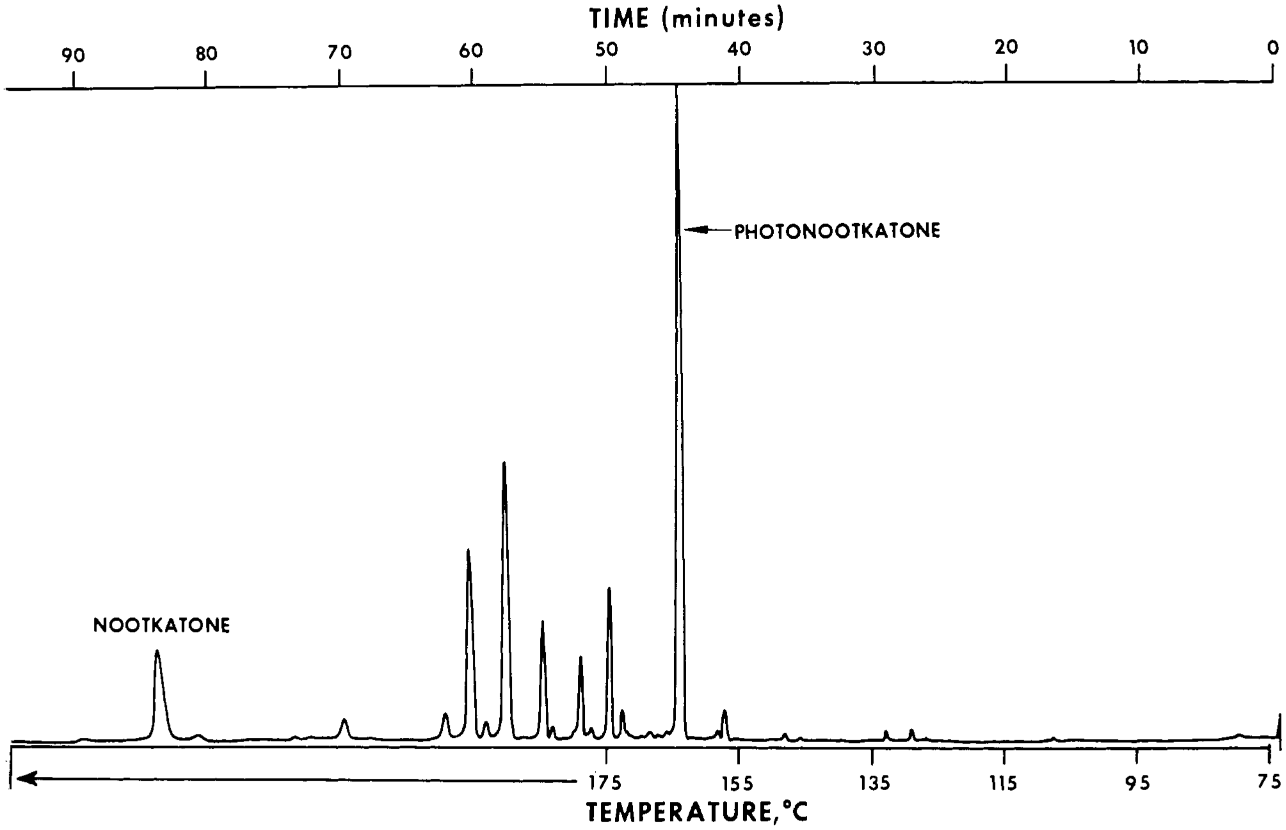


Figure 1. Gas chromatogram of photolyzed nootkatone
75-foot × 0.01-inch Igepal column

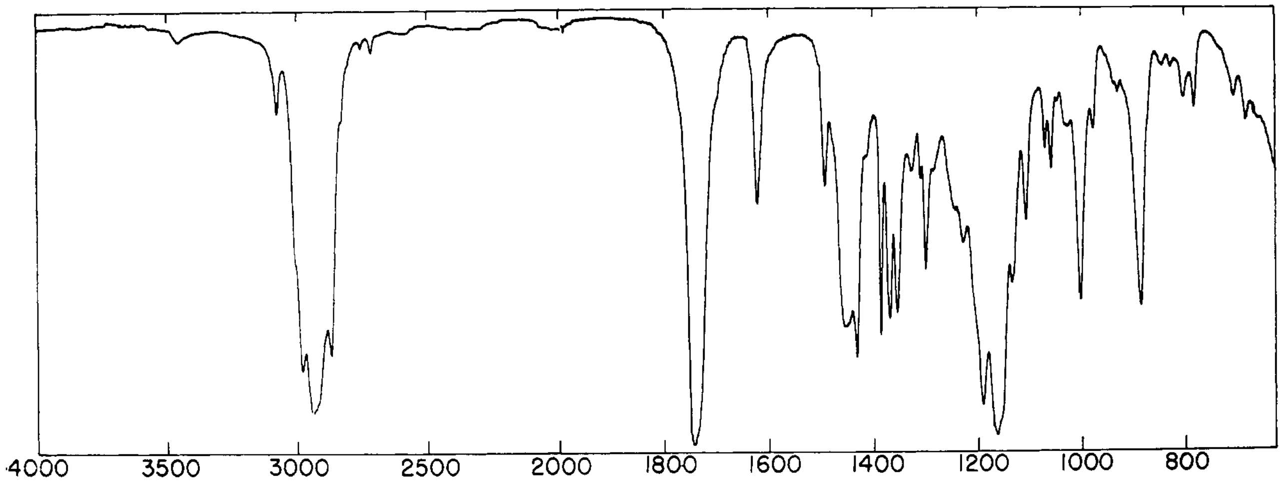


Figure 2. Infrared spectrum of photonootkatone (neat)

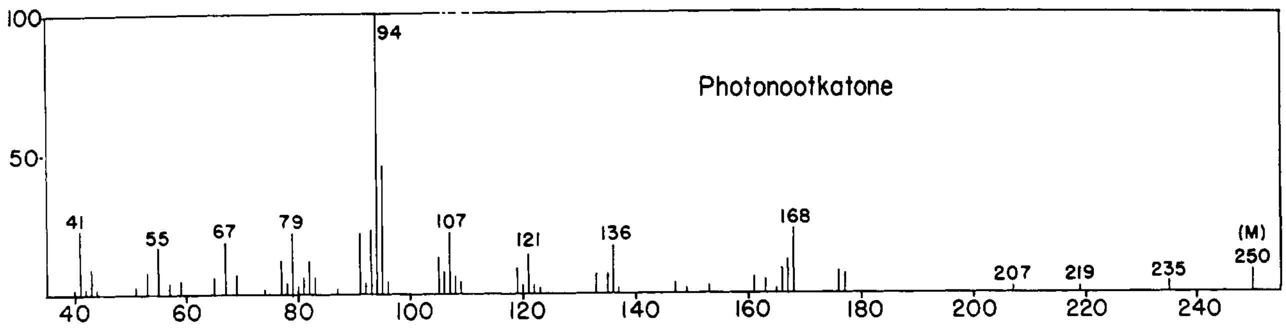


Figure 3. Mass spectrum of photonootkatone

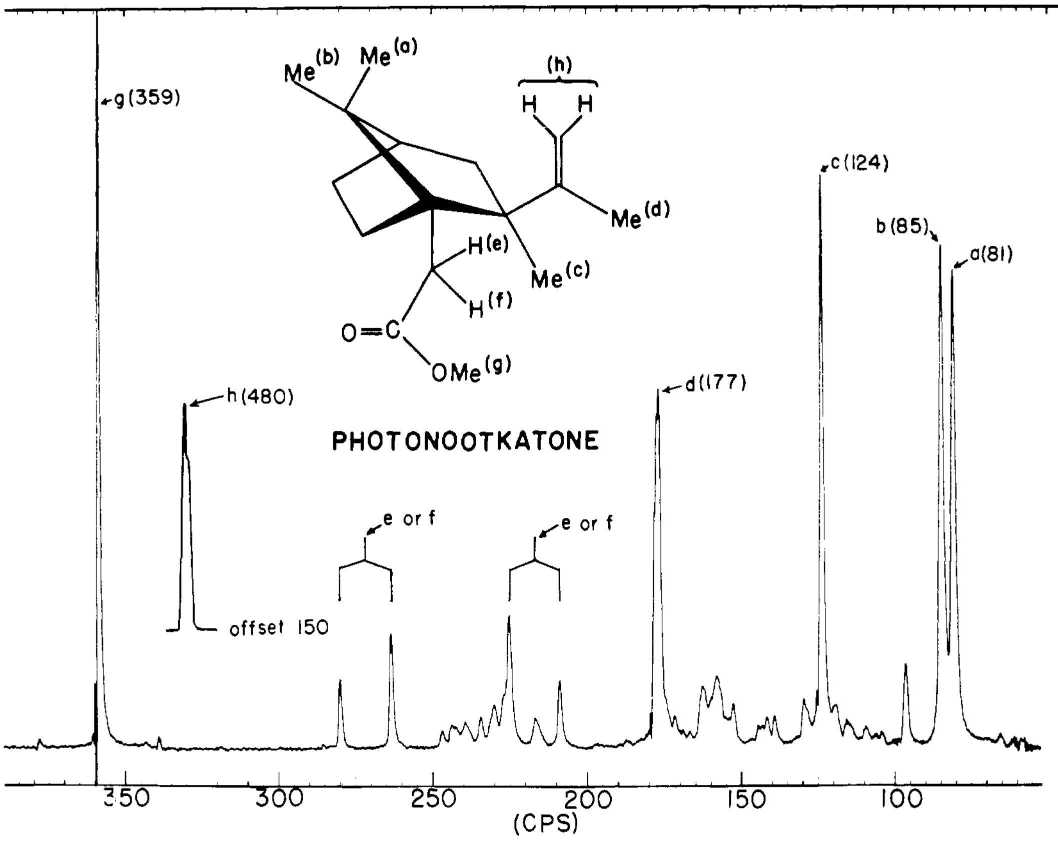


Figure 4. NMR spectrum of photonootkatone at 100 MHz.
Assignments relative to TMS as an internal standard

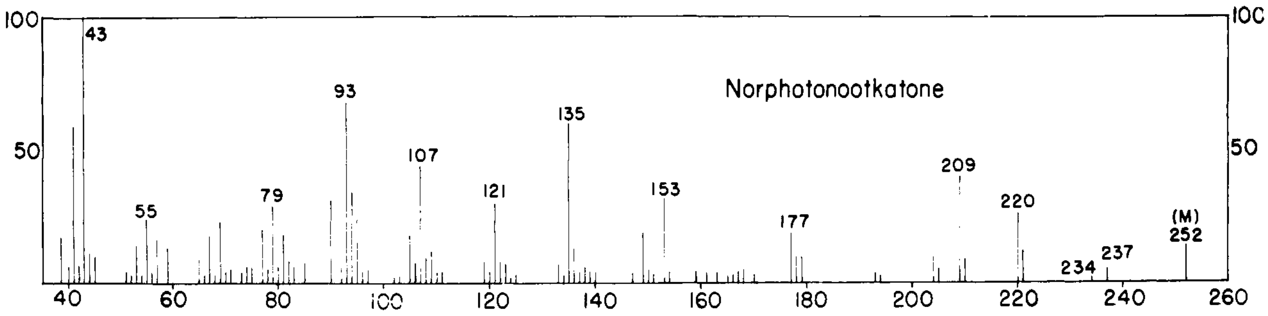
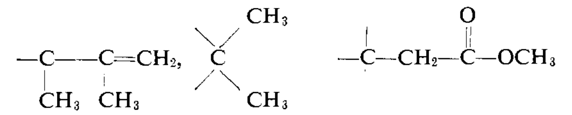


Figure 5. Mass spectrum of norphotonootkatone

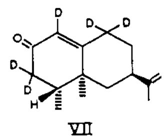
3 *H*) of photonootkatone has been replaced with a singlet (3 *H*) at 207 c.p.s. in norphotonootkatone, consistent with the formation of a methyl ketone.

Evidence presented thus far points rather conclusively to the presence of the following groups in photonootkatone:



$$-\underset{CH_3}{\underset{|}{C}}-\underset{CH_3}{\underset{|}{C}}{=}CH_2,\quad {}^{\diagdown}_{\diagup}C^{\diagup CH_3}_{\diagdown CH_3}\quad -\overset{|}{C}-CH_2-\overset{O}{\overset{\|}{C}}-OCH_3$$

The presence of an isopropenyl group attached to a tertiary center, unlike nootkatone, made it mandatory to determine if this group is the same one as in nootkatone or originates from some other part of the molecule. Deuteration of nootkatone by exchange with deuterium oxide gave nootkatone-d_5 (VII), in which the location of deuterium was



VII

confirmed by NMR spectroscopy. Irradiation of VII in methanol and isolation of the photoproduct gave photonootkatone-d_5. The NMR spectrum of the photoproduct still showed a vinyl methyl and an allyl methyl group, but the vinyl protons were replaced by deuterium. Infrared spectroscopy also showed the presence of an isopropenyl group. Apparently, the isopropenyl group in photonootkatone is not the same as in nootkatone.

Photonootkatone contains four "units" of unsaturation (molecular weight 250), with the carbonyl group and

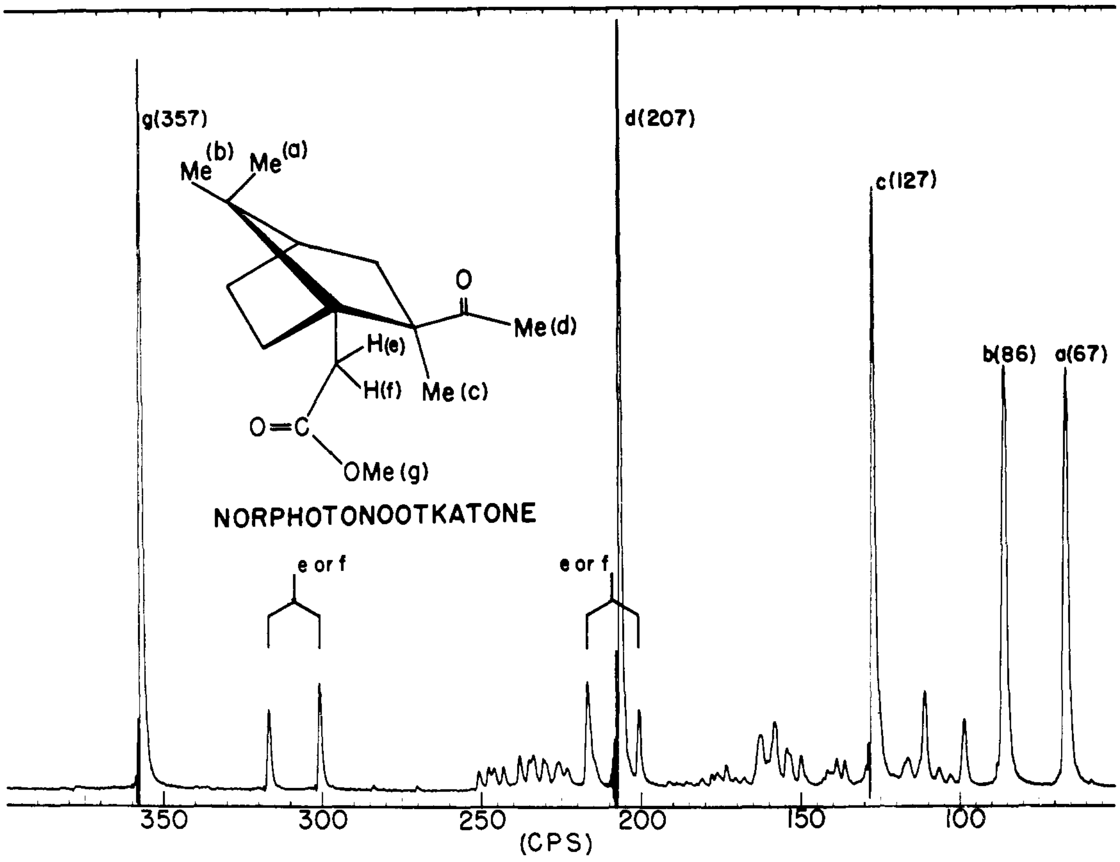
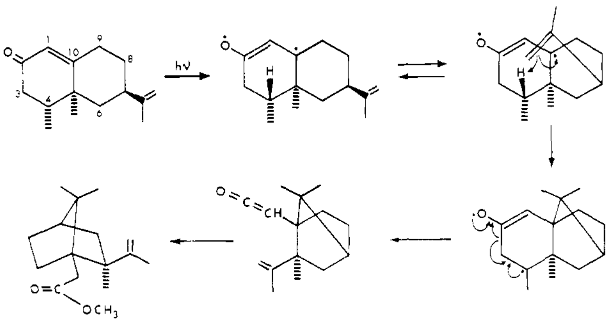


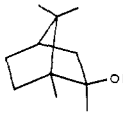
Figure 6. NMR spectrum of norphotonootkatone at 100 MHz.

double bond accounting for two of them. The remainder may be accounted for by two rings. Since apparently the isopropenyl group in nootkatone has been involved in the reaction, it becomes expedient to consider a bicyclo ring system, similar to carvonecamphor, to account for the two rings—i.e., an intramolecular cyclization. However, the reaction cannot be entirely analogous, because the product would not have the required number of methyl groups and would contain too many rings. To account for the data presented thus far, the following mechanistic scheme is proposed, leading to photonootkatone (VIII).



VIII

A molecular model of nootkatone in which ring B is in a boat form allows the isolated double bond to approach C-10 and the β-hydrogen on C-4. Bond formation and abstraction of hydrogen from C-4 may or may not be concerted. As can be seen from the proposed scheme, the structure of photonootkatone and mechanistic pathway accounts for all of the data. However, additional evidence was desired for the presence of a bicyclo[2.2.1]heptane ring system. An investigation of the Raman spectra of various substituted and unsubstituted bicyclo systems was initiated and, as was expected, an intense band appears in the C—C stretching region which may be ascribed to the symmetrical breathing mode of the ring system. The band, in both intensity and position, appears to be susceptible to substitution on the ring and to the type of ring system—for instance, camphor gives an intense band at 648 Δ cm.$^{-1}$, compound IX has an intense band at 638 Δ cm.$^{-1}$, and photonootkatone (VIII) gives a band at 613 Δ cm.$^{-1}$ (Figure 7). A more detailed and comprehensive study of the Raman spectra of bicyclo systems will be the subject of a future publication. The similarity of the Raman spectra of photonootkatone (Figure 7) and its derivatives to the substituted bicyclo[2.2.1]heptanes supports the proposed structure of photonootkatone (VIII).



IX

In addition to the close similarities in the Raman spectra, the NMR spectrum of photonootkatone bears many similarities to camphor and its derivatives—for instance, the *gem*-dimethyl group of IX resonates at 82 and 85 c.p.s.

The structure of norphotonootkatone then becomes X,

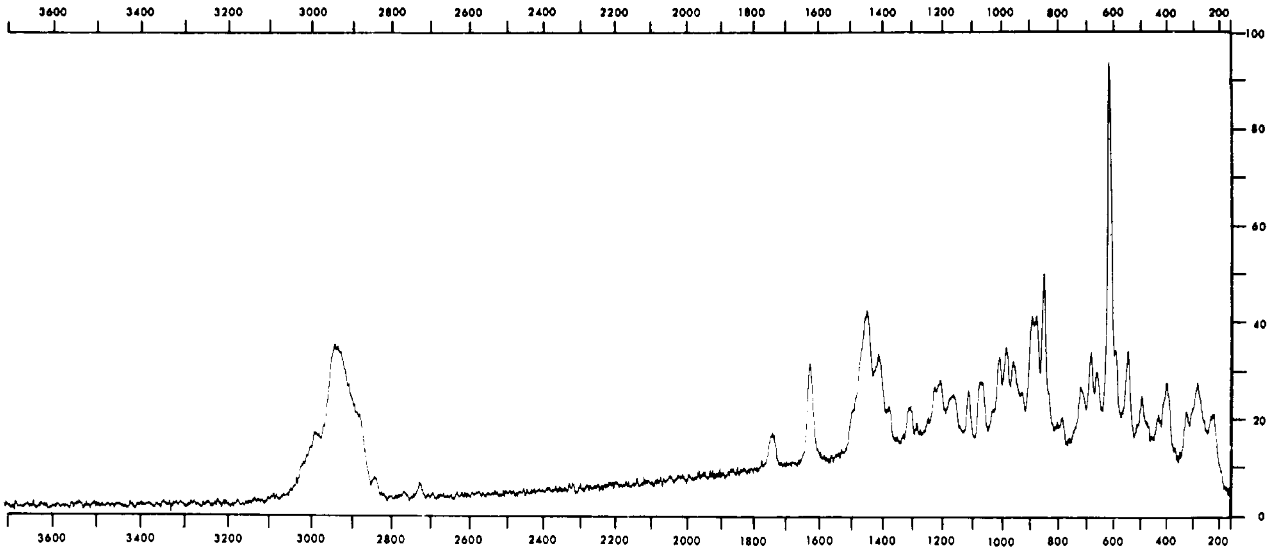
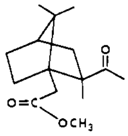


Figure 7. Raman spectrum of photonootkatone

Slit width 10 cm.$^{-1}$, time constant 2.8 seconds, scan speed 0.9 cm.$^{-1}$ per second, cell volume < 0.5 μl., laser power 9.5 mw.

in which the spectroscopic data are consistent. Two features of the NMR spectrum are significant. The singlet at 81 c.p.s. in photonootkatone has moved upfield to 67



X

c.p.s. in norphotonootkatone and the doublet with center at 272 c.p.s. in photonootkatone has moved downfield to 309 c.p.s. The high-field singlet has been assigned to one of the methyls of the *gem*-dimethyl group and if it lies in the shielding vicinity of the carbonyl, thus being shifted upfield, the authors would expect to find one of the protons of the methylene group adjacent to the ester moiety in the deshielding region and be shifted downfield (Jackman, 1959). The simultaneous upfield shift of the methyl group and downfield shift of the methylene proton further substantiate structures VIII and X for photonootkatone and norphotonootkatone, respectively. However, photonootkatone has not been converted to a known compound or synthesized and hence the structure must be considered provisional rather than rigorous.

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