Function A: $pKa' = 0.042\ (\%DMF) + 5.25$ (Eq. 2a)

Function C: $pKa' = 0.023\ (\%DMF) + 8.00$ (Eq. 2c)

Some of the literature data on pKa' (1–4) for the various tetracyclines in %DMF–$\%H_2O$ solutions are also plotted in Figs. 1 and 2 for comparison. It is apparent that the aureomycin and terramycin tetracyclines have lower pKa'-values but a similar relation with %DMF so that Eqs. 1 and 2 may be used as satisfactory estimates of pKa'-variation with %DMF—the same slopes but slightly lower intercepts.

A pKa' of 7.35 in 80%DMF–20%H_2O was observed for 10-benzenesulfonyltetracyclinonitrile. (The nitrile is substituted for the carboxamido group in *Ic*.) This value is consistent with the previously reported pKa' 6.9 at 50%DMF (2,4) for 10-benzenesulfonylterramycinonitrile which was assigned to the dimethylammonium ion. Addition of HCl to 10-benzenesulfonyltetracyclinonitrile showed no titratable group below pH 5 and above pH 2.0. This was consistent with the premise of highly increased acidity of the zwitterionic enolic system with nitril derivatives similar to but more acid than A in I(4).

The anhydrodesdimethylaminotetracycline, III, was observed to have two pKa'-values, pKa_1 = 8.06 (94%DMF), pKa_2 = 10.26 (91%DMF). This compound is analogous to anhydrodesdimethylaminoterramycin previously reported (2).

Now the pKa'-values of anhydroterramycin in water can be given (2) as 3.8 for A, 5.5 for C, and 7.2 for B, where the analogous functionalities are given in I. Since a 1,8-dihydroxybenzophthalide system has a pKa of 4.7 (2), the 5.5 pKa can possibly be assigned to the similar group C in anhydroterramycin; the 7.2 pKa' is consistent with the dimethylamine grouping B, zwitterionic with the carboxamide grouping A.

Removal of the dimethylamino group may consistently decrease the acidity of the carboxamido-enol complex A. It is noted that the pKa' 4.3 is elevated to 7.4, Δ pKa' = 3.1 in 50%DMF, and the pKa' 5.3 is elevated to 9.3, Δ pKa' = 4.0 in 95%DMF, for the change from tetracycline to desdimethylaminotetracycline (Figs. 1 and 2). Using these Δ pKa'-values, it can be predicted that on the insertion of a dimethylamino group into anhydrodesdimethylamino tetracycline III, the now zwitterionic carboxamide-enol complex could be assigned either the pKa'-values of 10.3 − 4.0 = 6.3, or 8.1 − 4.0 = 4.1 at 95%DMF. Because the derived pKa' of 6.3 for 95%DMF is the more consistent of these two possible values with the pKa' of the carboxamido-enol complex of tetracycline in 95%DMF, *i.e.*, 5.4 implies that the pKa_2 = 10.3 (94%DMF) is better assigned to the carboxamido grouping A in III, and the pKa_1 = 8.1 (94%DMF) can be assigned to the dihydroxynapthalene grouping C in III.

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Effect of Zinc Oxide Catalyzed Peroxide on Sterols of Almond, Cottonseed, and Olive Oils

By M. WAFIK GOUDAH and EARL P. GUTH

The phytosterols of almond, cottonseed, and olive oils undergo changes in structure when the oils are irradiated in both ultraviolet and sunlight with zinc oxide. The changes in the phytosterol molecules of the oils indicate a probability that a similar change could occur in the sterols of the skin. These changes might produce a molecule with anti-inflammation activity, thus providing an explanation for the dermatological effect of zinc oxide external preparations.

THE CATALYTIC ACTIVITY of zinc oxide in the photochemical formation of hydrogen peroxide has been confirmed by many workers (1, 2). The pharmaceutical aspects of the phenomenon have also been investigated by Guth, Reese, Mathias, Minardi, Blubaugh, Young, and Lozada (3–8).

Lozada and Guth (8) showed that when zinc oxide-containing ointments were irradiated with ultraviolet light in the presence of water and air the measurable peroxide was less or nonexistant in systems that contained cholesterol or other sterols. This was particulary true when wool fat or hydrophilic petrolatum were components of the ointment base. This lack of peroxide was accounted for by the effect on the sterol molecule.

Infrared spectrographic studies showed that the β-hydroxyl group on cholesterol was oxidized to a carbonyl group. There was also evidence that the double bond shifted from the 5:6 position to the 4:5 position.

With this as a background, a study of the effect of zinc oxide catalyzed peroxide on the sterols in

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olive oil, expressed almond oil, and cottonseed oil was undertaken.

EXPERIMENTAL

Calamine liniment, N.F. IX, was selected as the basic formula for the study. Zinc oxide "Baker" 3.2 Gm. was weighed into 1000-ml. Pyrex beakers, and 100 ml. of 0.2 *M* sodium formate solution was added. The contents were shaken by hand rotation. Ten milliliters of oil was added and the beakers (two at a time) were placed under a Dazor floating fixture ultraviolet lamp, model N. U-58 equipped with a General Electric VA-3 360 watt quartz photo chemical "Ulvirac" lamp.

Metal stirrers driven by Power-Stir model 58 motor, Eberbach Corporation, were used. The stirring was kept on the low speed indicated on the motor and was maintained through a 30-minute period of irradiation. The irradiation unit was completely enclosed within aluminum sheeting.

The phytosterols of each oil were isolated before and after each sample had been irradiated by the following method (9).

The sample of the oil was saponified with 2 *N* alcoholic potassium hydroxide under a reflux condenser. After saponification, most of the alcohol was removed by evaporation. The residual soap was dissolved in hot water and transferred to a separator. The flask was rinsed with hot water, and the rinsing was added to the funnel. After cooling, the unsaponifiable matter was extracted with three successive amounts of ethyl ether. The ethereal solutions were combined and washed with a small quantity of water to remove any residual soap. The ether was removed by distillation, and the residue was dried at 100°.

The unsaponifiable matter was dissolved in ethyl ether, and the solution was transferred to a small porcelain dish. The ether was allowed to evaporate spontaneously, the mass was dried on a water bath and dissolved (after cooling) in the smallest possible quantity of absolute alcohol. The phytosterols were allowed to crystallize from this solution.

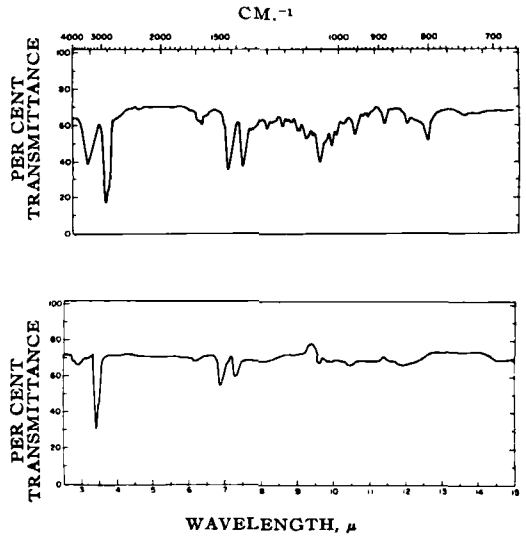

Fig. 1.—Infrared spectra of phytosterols in olive oil (top); in irradiated olive oil (bottom).

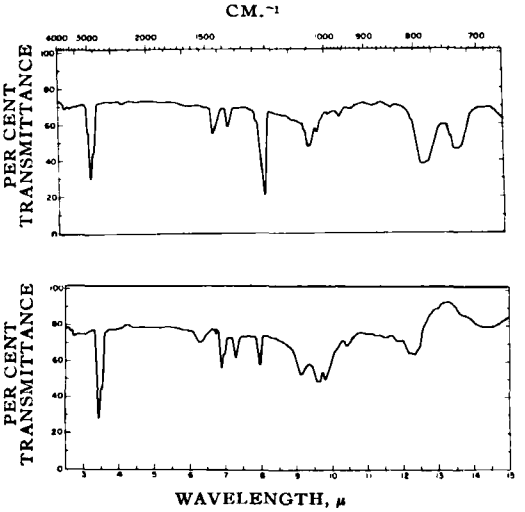

Fig. 2.—Infrared spectra of phytosterols in almond oil (top); in irradiated almond oil (bottom).

The sterols were dissolved in chloroform, and infrared spectrographs were determined in the Perkin–Elmer Infracord (Figs. 1–3). The phytosterols of cottonseed oil were also extracted after a mixture of zinc oxide and the oil had been exposed to the sunlight for 6 hours. The infrared spectra of the extracted phytosterols were determined and are shown in Fig. 3.

RESULTS AND DISCUSSION

The infrared spectra of the phytosterols of the oils isolated before irradiation showed a region from 4000 cm.$^{-1}$ (Figs. 1–3) which is characteristic of the functional groups in the molecule, while the "fingerprint" region is between 1350 cm.$^{-1}$ and 650 cm.$^{-1}$.

Absorption regions between 1055 cm.$^{-1}$ and 950 cm.$^{-1}$ are associated with the hydroxyl group in the sterol molecule. The absorption bands between 1464 cm.$^{-1}$ and 1375 cm.$^{-1}$ are characteristic of methylene group and angular methyl group. The 5:6 double bond gives bands at 840 cm.$^{-1}$ and 800 cm.$^{-1}$ (10). The region between 650 cm.$^{-1}$ and 800 cm.$^{-1}$ is also characteristic of all types of double bonds (11). All other absorption bands are "fingerprints" of the molecule.

The infrared spectra of the phytosterols of olive oil after it has been irradiated showed that variations occurred in the molecule. Bands due to the hydroxyl methyl group and the methylene group, although becoming reduced in intensity, are still present. The "fingerprint" bands showed a notable change. A very pronounced change is indicated by the disappearance of the double bond bands at 800 cm.$^{-1}$ and 840 cm.$^{-1}$.

The infrared spectra of the phytosterols of expressed almond oil after irradiation showed that certain groups disappeared and others shifted. The band at 1200 cm.$^{-1}$ was shifted to 1250 cm.$^{-1}$. As in the case of olive oil, the double bond region between 800 cm.$^{-1}$ and 650 cm.$^{-1}$ disappeared. Bands due to hydroxyl, methyl, and methylene groups were still present.

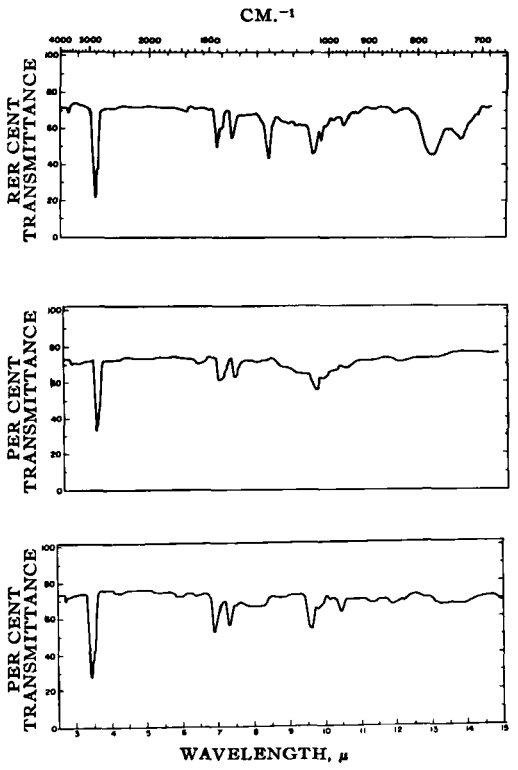

Fig. 3.—Infrared spectra of phytosterols in cottonseed oil (top); in irradiated cottonseed oil (middle); in 6 hour sunlight-exposed cottonseed oil (bottom).

Phytosterols of irradiated cottonseed oil still show bands of the hydroxyl, methyl, and methylene groups but of lessened intensity. The strong bands at 1200 cm.$^{-1}$ disappeared, and great changes in the "fingerprints" occurred. Also, (as with olive oil and almond oil) the double bond region between 800 cm.$^{-1}$ and 650 cm.$^{-1}$ disappeared.

When cottonseed oil, zinc oxide, sodium formate, and water were exposed to sunlight for 6 hours, changes also occurred in the phytosterol molecules. The changes are almost as identical as the changes in the oil irradiated under the ultraviolet lamp with the zinc oxide and sodium formate solution. The hydroxyl band was of greater intensity than when cottonseed oil was irradiated with ultraviolet light.

The disappearance of the double bond region from all the irradiated phytosterols, together with the other changes, indicates beyond doubt that the structure of the phytosterol molecule undergoes changes when exposed to the peroxides produced by zinc oxide and ultraviolet light. In the presence of oxygen–containing compounds such as water and air, Payot (12) found cholesterol susceptible to radiations and oxidizes on and about the 5,6 double bond. On the basis of the infrared spectrum and Payot's findings, it could be said that certain changes occurred in the molecule that might involve oxidation on and about the 5,6 double bond. Since hydrogen peroxide and ultraviolet irradiation energy are both present in this system, a reaction involving radical mechanism initiated by the energy of the ultraviolet very likely occurred in the phytosterol molecule.

When the sterols isolated from the sun-exposed cottonseed oil were tested pharmacologically they showed absence of anti-inflammatory activity. However, the changes which occurred in the molecule could possibly be a step in a chain of reactions which would yield a molecule that has anti-inflammatory effect. Since it has been shown that the production of hydrogen peroxide by zinc oxide and ultraviolet light as well as sunlight can cause structural changes in the sterols of natural lipids, it might be possible that changes could be caused in the sterols of the skin. If this were so, it would be a more rational explanation of the pharmacology of zinc oxide than the statements usually found in standard pharmacological references to zinc oxide action.

CONCLUSIONS

The infrared spectra of the phytosterols before and after irradiation proved that the phytosterols of the oils undergo changes in structure when the latter was irradiated in both ultraviolet and sunlight with zinc oxide. The double bond breakage in the sterol molecule was among the changes that occurred in the molecule.

Sunlight caused the same change in the sterol molecule as did ultraviolet light irradiations. Preliminary pharmacological tests showed that the sterols isolated from the sun-exposed cottonseed oil had no anti-inflammatory activity.

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