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# PSORALENS AND SUBSTITUTED COUMARINS FROM EXPRESSED OIL OF LIME

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**Abstract**—Chromatography on silica gel columns isolated ten crystalline solids from expressed West Indian lime (*Citrus aurantifolia*) oil. Of the eight compounds identified, three were those previously reported by Caldwell and Jones,[1] i.e. 5-geranoxy-7-methoxycoumarin, 5,7-dimethoxycoumarin and 5,8-dimethoxypsoralen (isopimpinellin). The following new compounds were identified: 5-geranoxypsoralen (bergamottin), 5-γ,γ-dimethylallyloxypsoralen (isoimperatorin), 8-geranoxypsoralen, 8-γ,γ-dimethylallyloxypsoralen (imperatorin); and 5-methoxy-8-γ,γ-dimethylallyloxypsoralen (phellopterin). Two compounds were tentatively identified as 5-geranoxy-8-methoxypsoralen and oxypeucedanin hydrate.

## INTRODUCTION

DURING an investigation of the coumarin compounds in expressed lemon oil,[2] we needed authentic samples of 5,7-dimethoxycoumarin and 5-geranoxy-7-methoxycoumarin for comparison. Lime oil, reported to contain these compounds as well as 5,8-dimethoxypsoralen (isopimpinellin),[1] was accordingly separated by chromatography on silica gel columns in the manner described for lemon oil.[2]

Cold-pressed peel oil of lime has been found to bear a remarkable resemblance in chemical composition to cold-pressed lemon (*Citrus limon* spp.). The major aldehydes and monoterpene hydrocarbons are similar in both identity and relative concentration.[3, 4] The similarity is further borne out in the dissolved solid compounds, the psoralens and coumarins, the identification of which is the subject of this paper. Nine of these compounds are found in both oils. Lemon oil in addition contains 5-isopenteneoxy-7-methoxycoumarin and byakancelicin. Lime oil, on the other hand, contains isopimpinellin (5,8-di-methoxycoumarin) which was not found in lemon oil.[3] Grapefruit oil contains chiefly the one compound 7-geranoxycoumarin with small amounts of other substituted coumarins and psoralens not found in lemon and lime. Oil of Bergamot more nearly resembles lemon and lime in containing 5-isopenteneoxy-7-methoxy-coumarin, 5-geranoxy-7-methoxycoumarin, and 5-geranoxypsoralen. In addition it contains 5-methoxypsoralen which we did not find in lemon and lime. Seville orange oil and oils of Valencia and Navel oranges contained mainly methyl ethers of flavonoids. We found osthol and bergapten in Seville orange and a new compound auraptenol,[5] but none of the compounds found in lemon and lime. A summary of these findings comparing the various citrus oils appears in Table 1.

* Present address, see below.

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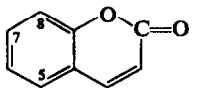
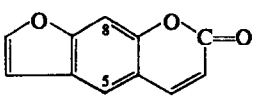
[1] A. G. CALDWELL and E. R. H. JONES, *J. Chem. Soc.* 540 (1945).
[2] W. L. STANLEY and S. H. VANNIER, *J. Am. Chem. Soc.* **79**, 3488 (1957).
[3] W. L. STANLEY, R. M. IKEDA, S. H. VANNIER and L. A. ROLLE, *J. Food Sci.* **26**, 43 (1961).
[4] W. L. STANLEY, R. M. IKEDA and SUSAN COOK, *Food Technol.* **15**, 381 (1961).
[5] W. L. STANLEY, A. C. WAISS, JR., R. E. LUNDIN and S. H. VANNIER, *Tetrahedron* **21**, 89 (1965).

The findings would tend to confirm Linnaeus' classification based on physical characteristics in which he divided the family Citrus *Rutaceae* into two species, i.e. *C. aurantium* containing sweet and bitter orange and the shaddock or grapefruit, and *C. medica* containing lemon, lime and citron.[6]

TABLE 1. SUBSTITUTED COUMARINS AND PSORALENS IN CITRUS OILS[18]



| | Lemon | Lime | Bergamot | Grapefruit | Seville orange |
|---|---|---|---|---|---|
| 7-Geranoxy | | | | × | |
| 7-Dihydroxygeranoxy- (Marmin?) | | | | × | |
| 7-Methoxy-8-isopentenyl- (Osthol) | | | | × | × |
| 5,7-Dimethoxy- (Limettin) | × | × | × | | |
| 5-Isopenteneoxy-7-methoxy | × | | | | |
| 5-Geranoxy-7-methoxy | × | × | × | | |
| 7-Methoxy-8- (2-hydroxy-3-methyl- 3-butenyl)- (Auraptenol) | | | | | × |
| **  ** | | | | | |
| 5-Psoralen (?) | | | | × | |
| 5-Psoralen (?) | | | | × | |
| 5-Methoxy (Bergapten) | | | × | | × |
| 5-Isopenteneoxy- (Isoimperatorin) | × | × | | | |
| 5-Geranoxy- (Bergamottin) | × | × | × | | |
| 5-Dihydroxyisopentanoxy- (Oxypeucedanin hydrate) | × | × | | | |
| 5-Hydroxy- (Bergaptol) | | | | × | |
| 8-Isopenteneoxy- (Imperatorin) | × | × | | | |
| 8-Geranoxy- | × | × | | | |
| 5,8-Dimethoxy- (Isopimpinellin) | | × | | | |
| 5-Methoxy-8-isopenteneoxy- (Phellopterin) | × | × | | | |
| 5-Methoxy-8-dihydroxy- isopentanoxy- (Byakangelicin) | × | | | × | |
| 5-Geranoxy-8-methoxy- | × | × | | | |

[6] *Sturtevant's Notes on Edible Plants* (Edited by U. P. HENDRICK), Vol. 2, Part II. 27th Annual Report, New York Agricultural Experiment Station (1919).

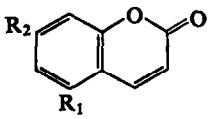
[18] W. L. STANLEY, *Aspects of Plant Phenolic Chemistry*, Proc. Symp. Plant Phenol. Group North America, p. 79. Toronto, Ontario, Canada (1963).

## RESULTS AND DISCUSSION

In all, we recovered ten crystalline solids from lime oil, of which three were the above-mentioned compounds. All ten compounds reacted negatively to the magnesium-hydrochloric acid test for flavones. The ultraviolet (u.v.) spectra of the compounds closely resembled those of the four types of coumarin and psoralen ethers in lemon oil,[2] i.e. 5,7-diethers of coumarin, 5- and 8-monoethers of psoralen, and 5,8-diethers of psoralen. Addition of caustic to their cold alcoholic solutions produced no bathochromic shift in the absorption spectra. Apparently the compounds did not contain free phenolic hydroxyl groups. The recovery data and $R_f$ values of the individual compounds in silica gel chromatostrips,[7] and their appearance on chromatostrips under u.v. light are given in Table 1.

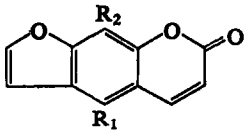
Compound I was shown to be 5-geranoxypsoralen (bergamottin) by direct comparison with authentic material from lemon oil.[2] Details of its identification appears in the Experimental section.

Compound II melted at 87–88°. Caldwell and Jones[1] report m.p. 86–87° for 5-geranoxy-7-methoxycoumarin from West Indian lime oil. The u.v. spectrum of II was virtually identical with the spectra given by them for 5,7-dimethoxy- and 5-geranoxy-7-methoxycoumarin. It was identical with a sample of 5-geranoxy-7-methoxycoumarin isolated from lemon oil.[2]




II. $R_1$=—$OC_{10}H_{17}$; $R_2$=—$OCH_3$; VIII. $R_1$=$R_2$=$OCH_3$; XII. $R_1$=OH; $R_2$=$OCH_3$.

Compound III, melted at 74·5–75·5°. Its u.v. spectrum was, with one exception, virtually identical with the spectra of the 5,8-diethers of psoralen, viz., byakangelicin,[2] 5-methoxy-8-$\gamma,\gamma$-dimethylallyloxypsoralen (phellopterin) (VII), and 5,8-dimethoxypsoralen (isopimpinellin) (IX). Whereas, in the spectra of the latter three compounds there was a broadening of the peak at about 270 m$\mu$, in the spectrum of III the corresponding peak was a sharp spike shifted slightly to lower wave length (268 m$\mu$) (Fig. 1). The methoxyl and elemental analyses of III satisfied the requirements of a diether of psoralen having a methyl and a geranyl group.




I. $R_1$=$OC_{10}H_{17}$; $R_2$=H; III. $R_1$=—$OC_{10}H_{17}$; $R_2$=—$OCH_3$; IV. $R_1$=—$OCH_2CH$=$C(CH_3)_2$; $R_2$=H; V. $R_1$=H; $R_2$=—$OC_{10}H_{17}$; VI. $R_1$=H; $R_2$=$OCH_2CH$=$C(CH_3)_2$; VII. $R_1$=—$OCH_3$; $R_2$=—O—$CH_2CH$=$C(CH_3)_2$; IX. $R_1$=$R_2$=—$OCH_3$; X. $R_1$=O—$CH_2$—CHOH—$COH(CH_3)_2$; $R_2$=H; XI. $R_1$=OH; $R_2$=H; XIII. $R_1$=—OH; $R_2$=—$OCH_3$; XIV. $R_1$=—$OCH_3$; $R_2$=—OH.

Compound III was assigned the structure of 5-geranoxy-8-methoxypsoralen on consideration of the dissimilarity of its u.v. spectrum from that of byakangelicin and phellopterin mentioned above and the following further observations. III was cleaved with acid to obtain the phenol (XIII), m.p. about 270° with decomposition. The acetate of XIII was prepared

[7] G. M. MILLER and J. G. KIRCHNER, *Anal. Chem.* **26**, 2002 (1954).

m.p. 207–209·5°. Briggs and Cambie[8] report the melting point of the isomeric phenol (in their nomenclature 8-hydroxy-5-methoxypsoralen, or in the more precise identification of Kincl *et al.*,[9] 9-hydroxy-4-methoxyfurano(3,2-g)benzopyran-7-one (II)) to be 221·5–222° and of the corresponding acetate, 180–181°. They also report the infra-red (i.r.) and u.v. spectra of the phenol. Phenol (XIV) obtained by acid cleavage of phellopterin (VII) and byakangelicin matched these physical properties satisfactorily. The u.v. spectra of XIII and XIV and the spectra of the two compounds in alkali are compared in Fig. 2. The i.r. spectrum of XIII deviates markedly from that reported for 5-methoxy-8-hydroxypsoralen.[8] The

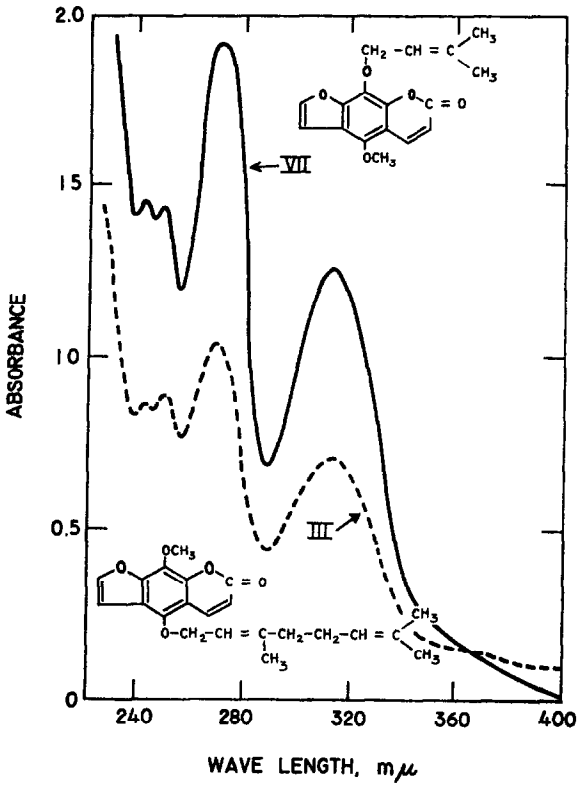



FIG. 1. U.V.-ABSORPTION SPECTRA 5,8-DIETHERS OF PSORALEN.
..... Compound III. ——— Compound VII.

5-position of the hydroxyl group was confirmed by the close resemblance of the u.v. spectrum of the acetate with that of 8-methoxypsoralen (Fig. 3). III has, therefore, been tentatively identified as 5-geranoxy-8-methoxypsoralen.

The u.v. spectrum of IV was virtually identical with that of 5-geranoxypsoralen (I). Its melting point, 110–110·5°, and elemental analyses suggested that it was 5-$\gamma,\gamma$-dimethylallyloxypsoralen (isoimperatorin). Späth and Kahovec[10] report the m.p. 109° for this compound. IV was cleaved with acid and the phenol was identical with XI obtained by similar cleavage

[8] L. H. BRIGGS and R. C. CAMBIE, *Tetrahedron* **2**, 256 (1958).
[9] F. A. KINCL, R. ROMO, G. ROSENKRANZ and F. SONDHEIMER, *J. Chem. Soc.* 4163 (1956).
[10] E. SPÄTH and L. KAHOVEC, *Ber. Deut. Chem. Ges.* **66**, 1146 (1933).

of I. Isoimperatorin was prepared by allowing 1-bromo-3-methylbutene-2 with anhydrous potassium carbonate to react with 5-hydroxypsoralen (from I) in acetone. The product could only be recovered by chromatography on silicic acid. Späth and Dobrovolny[11] also experienced some difficulty in preparing this ether. The mixture melting point of the synthetic isoimperatorin and IV was not depressed and the i.r. spectra of the two compounds were identical.

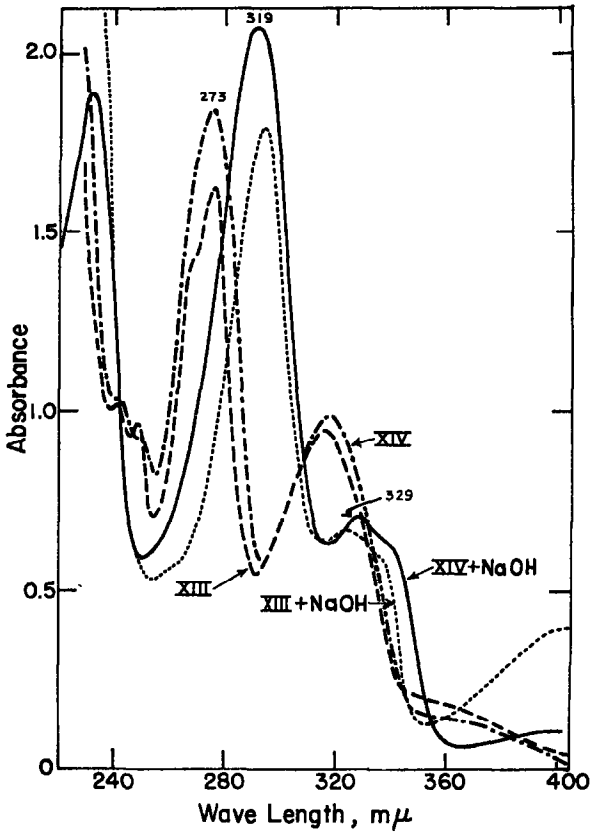



FIG. 2. U.V.-ABSORPTION SPECTRA OF METHOXYPSORALEN PHENOLS IN ETHANOL.
– – – – Compound XIII.
. . . . XIII with added sodium hydroxide.
–·–·– Compound XIV.
——— XIV with added sodium hydroxide.

Compound V was found to be identical with 8-geranoxypsoralen previously reported in lemon oil[2] by mixture melting point and comparison of i.r. and u.v. spectra. A small sample of V was ozonized in acetic acid and the products recovered by steam distillation. However, only the 2,4-dinitrophenylhydrazone of acetone could be identified.

Compound VI was found by elemental analysis to have the same composition as IV. Its u.v. spectrum was virtually identical with that of 8-geranoxypsoralen (V). VI was therefore concluded to be 5-$\gamma,\gamma$-dimethylallyloxypsoralen (imperatorin). Its identity was established

[11] E. SPÄTH and E. DOBROVOLNY, *Ber. Deut. Chem. Ges.* **72**, 52 (1939).

by mixture melting point and comparison of i.r. spectra with an authentic sample of imperatorin.[12]

The u.v. spectrum of VII was virtually identical with the spectra of byakangelicin and isopimpinellin (IX). The spectral evidence, elemental analyses, and melting point (104–104·5°) confirmed the supposition that VII was 5-methoxy-8-$\gamma,\gamma$-dimethylallyloxypsoralen (phellopterin) for which Noguchi and Kawanami report the melting point 102°.[13] VII was cleaved with acid and a phenol (XIV) obtained which melted at 218–221°. Briggs and Cambie[8] reported for 5-methoxy-8-hydroxypsoralen the melting point 221·5–222° and gave a tabulation of the absorption maxima in the i.r. and u.v. regions. These match the spectra obtained for XII with the exception of two peaks in the i.r. which may represent some impurity in the

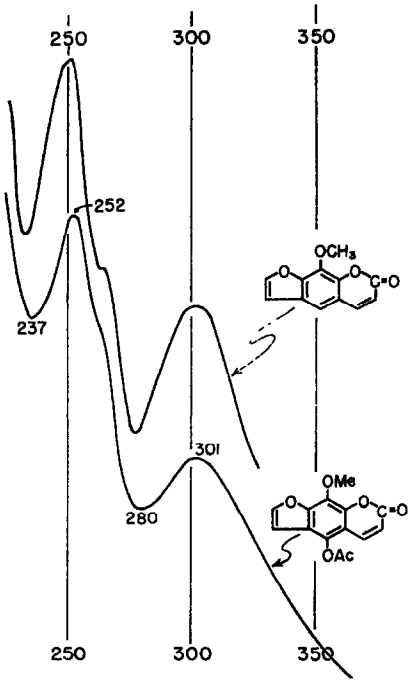



FIG. 3. U.V. SPECTRA OF ACETATE OF XIII AND 8-METHOXYPSORALEN.

product from lime oil. The acetate of XIV melted at 177–179° (lit. 180–181°).[8] As a further check, byakangelicin was cleaved with acid and the resulting phenol and its acetate compared with XIV and the corresponding acetate. The u.v. spectra of the phenols and bathochromic shift resulting from the addition of caustic and the u.v. spectra of the acetates were identical. The u.v. spectrum of the acetate resembled that of 5-methoxypsoralen (Fig. 4), confirming that the hydroxyl generated by acid cleavage of VII was at the 8-position. The mixture melting points of the corresponding compounds were not depressed. It was, therefore, concluded that VII is indeed 5-methoxy-8-$\gamma,\gamma$-dimethylallyloxypsoralen.

The u.v. spectrum of VIII was virtually identical with that of II. It emitted an intense blue fluorescence under u.v. The methoxyl and elemental analyses of VIII suggested that it was

[12] An authentic sample of imperatorin was kindly supplied by Professor T. O. Soine, University of Minnesota.
[13] T. NOGUTI and M. KAWANAMI, *J. Pharm. Soc. Japan* **60**, 57 (1940); *Chem. Abstr.* **34**, 3717 (1940).

5,7-dimethoxycoumarin (limettin), previously reported in West Indian lime oil.[1] The i.r. spectrum of VIII was identical with the spectra of limettin from lemon oil and limettin prepared in methylation of 5-hydroxy-7-methoxycoumarin,[2] and the mixture melting points were not depressed. Compound IX was bright yellow. Its u.v. spectrum was virtually identical with the spectra of VII and of byakangelicin. From its methoxyl and elemental analyses and melting point (150–151°) IX was judged to be isopimpinellin, previously reported in West Indian lime oil.[1]

The u.v. spectrum of X resembled closely the spectra of I and IV with one exception, namely, the peaks at 259 and 268 mμ were higher. Compound X melted at 129–132° (for oxypeucedanin hydrate Späth and Klager report 134°,[14] and Butenandt and Marten 136°).[15] Acetylation of X with acetic anhydride resulted in a diacetate melting at 115–116°. Attempts

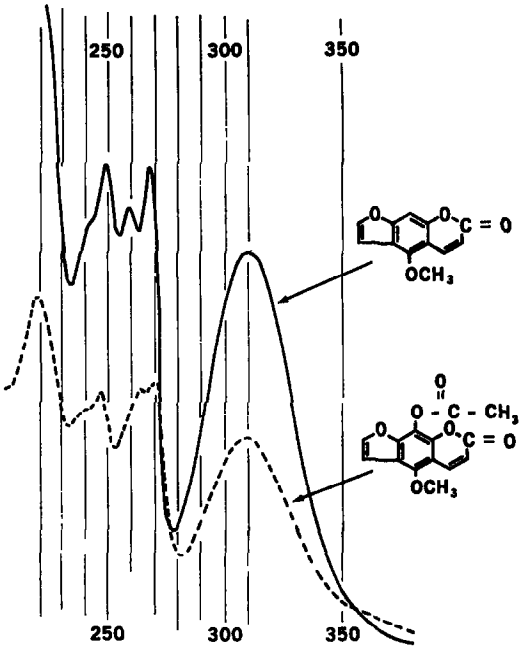



FIG. 4. U.V. SPECTRA OF ACETATE OF XIV AND 5-METHOXYPSORALEN.

to raise the melting point by additional recrystallizations were unsuccessful. For the diacetate of oxypeucedanin hydrate Späth and Klager report the melting point 132–133°.[14]

## EXPERIMENTAL

*Recovery of solids from lime oil.* Samples of cold-pressed West Indian lime oils were obtained from local processors and processors in Mexico. The local processors buy fruit primarily from Arizona. Samples of oil were separated in 200-g batches by chromatography on silica gel columns, 8 × 30 cm. The columns were prepared and the chromatograms developed in the manner described previously for lemon oil.[2] Eluate was collected in fractions which were tested by development on chromatostrips containing luminescent phosphors

[14] E. SPÄTH and K. KLAGER, *Ber. Deut. Chem. Ges.* **66**, 914 (1933).
[15] A. BUTENANDT and A. MARTEN, *Annalen* **495**, 187 (1932).

with ethyl acetate in hexane (25:75 v/v) as developing solvent. The developed chromatostrips were examined under u.v. light (maximum emission at 253 nm) for fluorescent compounds and for compounds capable of absorbing in the wave length of the exciting illumination. These latter compounds appeared as purple shadows on a yellow background. Tubes from the fraction collector that contained similar material (based on u.v. fluorescence, color tests, and $R_f$) were combined into thirty composites. With the exception of the first composite, they were reduced to a convenient volume (2–10 ml) by flashing off solvent in a rotary vacuum evaporator. The first composite containing limonene and other terpene hydrocarbons, which represented about 70 per cent of the original oil, was discarded. The concentrated composites were stored at 5°. Those depositing crystals were filtered and the crystalline products purified by recrystallization. A summary of the yields of these products, their $R_f$ values, and appearance under u.v. on chromatostrips appears in Table 2.

TABLE 2. *R* VALUES AND YIELD OF SOLID COMPOUNDS FROM LIME OIL*

| | $R_f$ value† | Recovery (mg/100 g oil) |
|---|---|---|
| 5-Geranoxypsoralen (I) | 0·68 | 3025 |
| 5-Geranoxy-7-methoxy-coumarin (II) | 0·64 | 1725 |
| 5-Geranoxy-8-methoxy-psoralen (III) | 0·59 | 945 |
| Isoimperatorin (IV) | 0·57 | 33 |
| 8-Geranoxypsoralen (V) | 0·40 | 105 |
| Imperatorin (VI) | 0·27 | 18 |
| Phellopterin (VII) | 0·25 | 11 |
| Limettin (VIII) | 0·25 | 464 |
| Isopimpinellin (IX) | 0·14 | 508 |
| Oxypeucedanin hydrate (X) | 0·01 | 25 |

* Under u.v. on chromatostrips that contained phosphors all these compounds were purple shadows except II and VIII that were blue fluorescences.

† $R_f$ values determined on silica gel chromatostrips by capillary ascent with 25% v/v ethyl acetate in hexane. Viewed under short wave length light (max. emission 253 nm).

*I.R. and Melting Points*

Samples were incorporated in potassium bromide discs for i.r. determinations. Melting points were uncorrected and were determined in a Kofler block.

*5-Geranoxypsoralen (I).* During concentration of composite 8, a copious crop of crystals precipitated which, after recrystallization from an ethyl acetate–hexane mixture, melted at 59–60°. Fraction 9 was shown by chromatostrip tests to be a mixture of I and II. The crystalline mixture from this fraction was recovered (0·258 g), but no attempt was made to separate the mixture. U.V. of I: $\lambda_{max}$ 245 (shoulder, log$\epsilon$ 4·22), 251 (4·27), 260 (4·21), 268 (4·20), and 310 nm (4·15). The addition of caustic did not shift the spectrum. The i.r. spectrum was identical with spectra of samples of 5-geranoxypsoralen from lemon[2] and bergamot[16] oils.

*Anal.* (Found: C, 74·7; H, 6·72. $C_{21}H_{22}O_4$ required: C, 74·5; H, 6·55%.) Ozonolysis of

[16] E. SPÄTH and P. KAINRATH, *Ber. Deut. Chem. Ges.* **70**, 2272 (1937).

I yielded acetone and levulinic aldehyde as shown by conversion to the 2,4-dinitrophenylhydrazones. The 2,4-DNPH derivatives melted at 125·5–126·5°, and 224–226°, respectively. The melting points of mixture with authentic derivatives of acetone and levulinic aldehyde (the latter obtained by the ozonolysis of geraniol) were not depressed.

*5-Hydroxypsoralen XI.* A portion of I (1·0068 g) was dissolved in glacial acetic acid (4 ml), $H_2SO_4$ (0·2 g) added, and the mixture stirred at room temperature for 1½ hr. A light tan precipitate was filtered off, washed with cold ethanol, and dried under vacuum overnight in a desiccator. The product (XI), colorless clumps of blades (468·8 mg) melted at 278–280° and mixture with material obtained similarly from lemon oil and bergamot oil gave no depression in melting point (lit. for 5-hydroxypsoralen, m.p. 275–278°[4]). U.V. $\lambda_{max}$ 243 (shoulder, log $\epsilon$ 4·13), 250 (4·20), 263 (shoulder, 4·19), 269 (4·27), 313 nm (4·14). With added sodium hydroxide u.v.: $\lambda_{max}$ 231 (shoulder, log $\epsilon$ 4·37), 234 (4·38), 288 (4·34), 323 nm (4·00). Minor irregularities also appeared at 260, 330, and 336 nm, and there was a broad peak at about 400 nm (log $\epsilon$ 3·76).

The acetate was prepared by refluxing with acetate anhydride acid freshly fused sodium acetate. The product was recrystallized from methanol yielding colorless needles (35 mg) melting at 169–172°. The mixture melting point with 5-acetoxypsoralen (m.p. 177–179°) prepared in the same manner from 5-geranoxypsoralen from lemon oil[2] was 173–175°.

*5-Geranoxy-7-methoxycoumarin (II).* During concentration of composite 10 a voluminous deposit of crystals appeared which was filtered off and recrystallized from ethyl acetate–hexane mixture to obtain pure 5-geranoxy-7-methoxycoumarin, colorless blades melting at 87–88°. Caldwell and Jones report the m.p. 86–87° for this material isolated from West Indian lime oil. The mixture melting point with material isolated from lemon oil[2] was not depressed and comparison of i.r. spectra confirmed that the compounds were identical. U.V.: $\lambda_{max}$ 247 (log $\epsilon$ 3·85), 256 (3·85), 327 nm (4·18). The addition of caustic did not shift the spectrum.

*Anal.* (Found: C, 73·2; H, 7·47; MeO—, 9·3. $C_{20}H_{24}O_4$ required: C, 73·1; H, 7·37: 1 MeO—, 9·4.)

*5-Geranoxy-8-methoxypsoralen (?) (III).* The concentrate from composite 11 on standing at 5° deposited crystals of III which were filtered off and washed on the filter with ethyl acetate–hexane mixture, m.p. 74·5–75·5° (18 mg). Composite 12 was tested with chromatostrips and found to contain two components, III and IV. The mixture was separated by rechromatographing on silica gel and yielded III (171 mg) and IV (isoimperatorin) (65 mg). U.V. of III: $\lambda_{max}$ 242 (log $\epsilon$ 4·18), 250 (4·19), 268 (4·28), 312 nm (4·07). There was no shift in spectrum on the addition of caustic.

*Anal.* (Found: C, 71·7; H, 6·70; MeO—, 8·7. $C_{22}H_{24}O_5$ required: C, 71·7; H, 6·57; 1 MeO—, 8·4.)

*5-Hydroxy-8-methoxypsoralen (?) (XIII).* A portion of III was cleaved with sulfuric acid in glacial acetic acid in the manner described for I. The resulting 5-hydroxy-8-methoxypsoralen melted at about 260° with decomposition. The mixture melting point with the phenol from VII was depressed. U.V.: $\lambda_{max}$ 224 (log $\epsilon$ 4·31), 241 (4·11), 248 (3·99), 268 (shoulder, 4·16), 275 (4·21), 316 nm (log $\epsilon$ 3·98). With added sodium hydroxide, u.v.: $\lambda_{max}$ 232 (log $\epsilon$ 4·28), 324 (3·83), 332 (shoulder, 3·81), 337 nm (3·78). The acetate was prepared by refluxing with excess acetic anhydride and freshly fused sodium acetate and recrystallized from ethanol, needles melting at 207–209·5°. $\lambda_{max}$ 252, 265 sh., 301 nm (8-MeO—Psor.; 250, 264 sh., 301 nm).

*Anal.* (Found: C, 61·3; H, 3·75. $C_{14}H_{10}O_6$ required: C, 61·3; H. 3·68.)

*Isoimperatorin (IV).* As described above for purification of III, rechromatography of composite 12 yielded two products. From the second of these was obtained IV (65 mg) which was filtered off and washed on the filter with hexane, m.p. 110–110·5°, clusters or nodules of fine needles. U.V.: $\lambda_{max}$ 243 (shoulder, log$\epsilon$ 4·14), 250 (4·19), 259 (4·14), 268 (4·13), 310 nm (4·09). The addition of caustic caused no shift in the spectrum.

*Anal.* (Found: C, 71·1; H, 5·34. $C_{16}H_{14}O_4$ required: C, 71·1; H, 5·22.)

*Preparation of isoimperatorin.* A portion of I (1·018 g) was dissolved in glacial acetic acid (5 ml) with stirring and to this mixture 0·05 ml conc. $H_2SO_4$ added. A yellow solid precipitated immediately. Stirring was continued for 10 min. The solid was filtered off and the residue washed on the filter with glacial acetic acid followed by several washings with distilled water. The yield of crude 5-hydroxypsoralen (m.p. 265–270°) was 0·450 g. A mixture of 5-hydroxypsoralen (0·102 g), 1-bromo-3-methylbutene-2,[17] and anhydrous $K_2CO_3$ in acetone was heated under reflux for 16 hr. The solvent was evaporated under nitrogen and the residue taken up in hexane–ethyl acetate mixture. A small amount of insoluble yellow material was filtered off and the filtrate placed on a silica gel column 2·3 × 15 cm long prepared as described for the initial oil separations. The column was developed with 10% v/v ethyl acetate in hexane and eluate collected in a clock-actuated fraction collector. The first fractions, which contained material appearing as dark spots on chromatostrips, were combined, evaporated to dryness, and crystallized from hexane–ethyl acetate, m.p. 105–108°. The mixture melting point with IV was not depressed and the i.r. spectra of the two compounds were identical.

*Cleavage of IV.* A small amount (30 mg) of IV was cleaved in glacial acetic acid containing sulfuric acid as in our preparation of isoimperatorin. The recovered solid was washed on the filter with water and cold ethanol, nodules of fine blades (8 mg) m.p. 257–260°. The mixture melting point with 5-hydroxypsoralen (m.p. 278–280°) which had been recrystallized from ethanol was 270–272°. U.V.: $\lambda_{max}$ 222, 243 (shoulder), 251, 262 (shoulder), 269, 313 nm. With alkali added, u.v.: $\lambda_{max}$ 230 (shoulder), 234, 288, 323 nm with minor irregularities at 232, 330, and 337 nm and a broad peak at about 400 nm. This spectrum is virtually qualitatively identical to that obtained for 5-hydroxypsoralen from I above. The log$\epsilon$ values were not determined.

8-*Geranoxypsoralen (V).* The concentrate from composite 15 on standing at 5° deposited crystals which, after recrystallization from hexane–ethyl acetate, melted at 58–59°. U.V.: $\lambda_{max}$ 244 (shoulder, log$\epsilon$ 4·31), 249 (4·33), 264 (shoulder, 4·15), 311 nm (4·04). The addition of caustic did not shift the spectrum. The mixture melting point with authentic material isolated from lemon oil[2] was not depressed and the i.r. spectra of the two compounds were identical.

*Anal.* (Found: C, 73·8; H, 6·57. $C_{21}H_{22}O_4$ required: C, 74·53; H. 6·55.)

A small sample of V was ozonized and the 2,4-DNPH of acetone recovered in the manner described above for I. However, levulinic aldehyde was not recovered from the ozonization mixture.

*Imperatorin (VI).* The concentrate from composite 18 on standing at 5° deposited crystals which were filtered off and recrystallized from ethyl acetate–hexane mixture, m.p. 104–104·5°. The mixture melting point with a known sample of imperatorin[12] (m.p. 97·5–101°) was 100–102°. A comparison of the i.r. spectra of the two compounds confirmed their identity. U.V.: $\lambda_{max}$ 245 (shoulder, log$\epsilon$ 4·31), 250 (4·33), 264 (shoulder, 4·11), 301 nm (4·05).

*Anal.* (Found: C, 70·8; H, 5·29. $C_{16}H_{14}O_4$ required: C, 71·1; H, 5·22.)

[17] H. STAUDINGER, W. KRESIS and W. SCHILT, *Helv. Chim. Acta* **5**, 743 (1922).

*Phellopterin* (*VII*). The concentrate from composite 19, on standing at 5°, deposited bulky prisms which were filtered off and washed on the filter with a small amount of ethyl acetate–hexane mixture, m.p. 104–104·5°. (Noguchi and Kawanami report m.p. 102° for phellopterin.)[10] U.V.: $\lambda_{max}$ 242 (log $\epsilon$ 4·14), 249 (4·14), 269 (4·26), 273 (shoulder which could be considered part of a broad peak, 4·26), 313 nm (4·07).

*Anal.* (Found: C, 67·9; H, 5·25; MeO—, 10·4. $C_{17}H_{16}O_5$ required: C, 68·0; H, 5·37; 1 MeO—, 10·33.)

*5-Methoxy-8-hydroxypsoralen* (*XIV*). A mixture of VII (71 mg) in glacial acetic acid (1 ml) containing 20 mg concentrated sulfuric acid was stirred at room temperature for 5 min. The bright yellow precipitate which began forming in 2 min was filtered, washed on the filter with acetic acid and distilled water, and recrystallized from ethanol (13 mg), m.p. 218–221°. U.V.: $\lambda_{max}$ 241 (shoulder, log $\epsilon$ 4·01), 250 (shoulder, 3·96), 273 (4·26), 317 nm (4·00). With caustic added, u.v.: $\lambda_{max}$ 292 (log $\epsilon$ 4·32), 328 (3·85), 340 (shoulder, 3·79), about 400 nm (3·04).

The i.r. spectrum of XIV matched that reported for 9-hydroxy-4-methoxyfurano(3,2-g) benzopyran-7-one by Briggs and Cambie[8] with the exception of additional peaks, one at 1160 on the side of the 1148 $cm^{-1}$ peak and at 838 on the side of the 833 $cm^{-1}$ peak. The peak at 746 was shifted to about 740 $cm^{-1}$. These additional peaks may result from differences in the preparation of the potassium bromide disc or from the presence of an impurity.

The acetate melted at 177–179° (Briggs and Cambie[8] report 180–181°). U.V.: $\lambda_{max}$ 222 (log $\epsilon$ 4·35), 240 (4·18), 247 (4·20), 263 (4·21), 270 (4·23), 310 nm (4·11).

*Anal.* (Found: C, 61·3; H, 3·75. $C_{14}H_{10}O_6$ required: C, 61·3; H, 3·68.)

*Cleavage of byakangelicin.* A portion of byakangelicin from lemon oil[2] (87 mg) was dissolved in glacial acetic acid (1 ml) containg 0·21 ml conc. $H_2SU_7$ and heated on the steam bath for 1 hr. When water was added (2–3 ml), a black precipitate appeared. The precipitate was filtered, washed with water, then with 5% $NaHCO_3$, and again with water. The precipitate was sublimed at below 1 mm Hg. The first sublimate, appearing at 190–200° was discarded; whereas, that appearing at 240° was collected, m.p. 210–215°. The mixture melting point with XIV from cleavage of VII (above) was 213–216°. The acetate was prepared according to the method described above, m.p. 177–181°. The mixture melting point with the acetate of XIV was not depressed (177–180°).

*Limettin* (*VIII*). During concentration of composite 20 a crystalline precipitate appeared. The product was filtered off and recrystallized from hexane containing about 20% v/v ethyl acetate, m.p. 147·5–149°. The mixture melting point with authentic material from lemon oil was not depressed. The i.r. spectra of the two compounds were identical. U.V.: $\lambda_{max}$ 246 (log $\epsilon$ 3·77), 255 (3·77), 326 nm (4·17). There was no shift in spectrum on the addition of caustic.

*Anal.* (Found: C, 64·0; H, 5·0; MeO—, 29·9. $C_{11}H_{10}O_4$ required: C, 64·1; H, 4·89; 2 MeO—, 30·10.)

*Isopimpinellin* (*IX*). During concentration of composite 21 a bright yellow crystalline product appeared. The mixture was allowed to stand at 5° and the solid filtered off and recrystallized from ethyl acetate–hexane mixture, yellow needles, m.p. 150–151° (lit. 148–151° and 147–148° from lime oil[1]). U.V.: $\lambda_{max}$ 242 (log $\epsilon$ 4·16), 248 (4·16), 269 (4·27), 273 (shoulder which could be considered part of a broad peak, 4·27), 312 nm (4·10). There was no shift in spectrum on the addition of caustic. The spectra were virtually identical with those obtained for VII (phellopterin) and byakangelicin[2] (cf. Fig. 1).

*Anal.* (Found: C, 63·4; H, 4·36; MeO—, 24·9. $C_{13}H_{10}O_5$ required: C, 63·4; H, 4·09; 2 MeO—, 25·1.)

*Oxypeucedanin hydrate* (*X*). The concentrate from composite 25, on standing at 5°, deposited light-tan prisms which were filtered off and recrystallized from ethanol, m.p. 129–132° (lit. for oxypeucedanin hydrate, 134°[11] and 136°[12]). U.V.: $\lambda_{max}$ 222 (log $\epsilon$ 4·35), 250 (4·22), 259 (4·18), 269 (4·20), 310 nm (4·15).

*Anal.* (Found: C, 63·1; H, 5·37. $C_{16}H_{16}O_6$ required: C, 63·2; H, 5·30.)

*Diacetate of oxypeucedanin hydrate.* This was prepared by refluxing for 2 hr with acetic anhydride and sodium acetate. The product was recrystallized from methanol, followed by recrystallization from petroleum ether–chloroform, m.p. 115–116°. U.V.: $\lambda_{max}$ 222 (log $\epsilon$ 4·37), 243 (shoulder, 4·23), 250 (4·29), 258 (4·27), 267 (4·23), 308 nm (4·17).

*Anal.* (Found: C, 61·8; H, 5·35. $C_{20}H_{20}O_8$ required: C, 61·9; H, 5·19.)

Späth and Klager[14] report for this acetate the m.p. 132–133° with correct analyses. We have not, however, been able to obtain their reported melting point, even when using the same solvents for crystallization (chloroform–petroleum ether).

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