

THE THIN LAYER CHROMATOGRAPHIC CHARACTERIZATION OF SOME PHENOLIC COMPOUNDS RELATED TO THE TOCOPHEROLS AND THEIR OXIDATION PRODUCTS

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In connection with other work concerning the oxidation products and metabolites of the tocopherols which is underway in this laboratory, it was necessary to investigate the chromatographic behavior of a number of model compounds. The silica gel thin-layer system was particularly well suited for the separation of these materials; the three best solvents used are shown in Table I, which summarizes the R_F values for 27 hydroquinones, hydroquinone diacetates, chromanols, dichromans, and various oxidation products of the α -tocopherol model compound (2,2,5,7,8-pentamethyl-6-hydroxychroman).

Three different spray reagents were used for development of the finished chromatograms; 60% sulfuric acid, followed by heating in an oven at 150°, as a general reagent which shows the location of most organic compounds, 5% potassium ferricyanide followed by 5% ferric chloride which shows compounds oxidized by ferricyanide ion with formation of Turnbull's blue¹, and neutral silver nitrate in acetone which generally detects the free phenolic hydroxyl compounds². The color reactions with these reagents are also shown in Table I.

The melting points are given for all compounds along with a reference to the literature melting point. In cases where there is a disagreement with the literature melting points, some other evidence is given to substantiate the compound's authenticity, *e.g.*, elementary analysis, nuclear magnetic resonance (NMR), infra red spectra, or derivatization. Several of the compounds have not previously been reported.

EXPERIMENTAL

The thin layer plates (8 × 8 in.) were prepared from "Silica-Gel G. according to Stahl," (Brinkmann Instruments Co., Great Neck, Long Island, N. Y.) by mixing 30 g of the dry powder with 60 ml of distilled water and applying to the glass plates with a 250 μ spreader. After air drying the plates were baked in an oven for 1 h at 110°.

All chromatograms were prepared by the ascending method at 20° in a solvent saturated atmosphere. The compounds (27 from ethanol solution) were spotted 2 cm from the bottom of the thin layer plate. The plate was submerged in solvent to a depth of 3 to 5 mm and the solvent allowed to run a distance of 15 cm. The running times for the 15 cm of solvent travel for the respective solvents were: chloroform, 47 min; benzene, 35 min; cyclohexane-tetrahydrofuran (THF) (1:1), 53 min.

TABLE I
CHARACTERIZATION OF SOME PHENOLIC COMPOUNDS RELATED TO TOCOPHEROLS AND THEIR OXIDATION PRODUCTS

| Compound | Reference | Melting points | | R _F | | | Spray reagents | | |
|--|-----------|----------------|---------|----------------|---------|--------------------------|--------------------------------|------------------------------------|-------------------|
| | | Literature | Found | Chloroform | Benzene | Cyclohexane THF (1:1) | H ₂ SO ₄ | K ₂ Fe(CN) ₆ | AgNO ₃ |
| Hydroquinones | | | | | | | | | |
| Unsubstituted | 4 | 172-3 | 172-174 | 00-04 | 00-04 | 43-47 | tan | blue | grey |
| 2-(CH ₃) | 5 | 124-125 | 127-129 | 00-05 | 00-04 | 45-50 | tan | blue | grey |
| 2,3-(CH ₃) ₂ | 6 | 221 | 225-226 | 02-06 | 00-04 | 49-54 | grey | blue | grey |
| 2,6-(CH ₃) ₂ | 7 | 149-151 | 145-147 | 05-08 | 02-06 | 51-56 | yellow | blue | grey |
| 2,5-(CH ₃) ₂ | 8 | 212 | 219-220 | 05-09 | 02-05 | 53-58 | tan | blue | grey |
| 2,3,5-(CH ₃) ₃ | 4 | 170 | 170-173 | 00-25 | 03-06 | — | yellow | blue | grey |
| 2,3,5,6-(CH ₃) ₄ | 9 | 220 | 229-230 | 00-30 | 03-05 | — | yellow | blue | grey |
| Hydroquinone diacetates^a | | | | | | | | | |
| Unsubstituted | 10 | 121-122 | 123-124 | 48-54 | 05-08 | 52-56 | tan | — | — |
| 2-(CH ₃) | 11 | 43-44 | 35-36 | 52-57 | 05-08 | 56-62 | tan | — | — |
| 2,3-(CH ₃) ₂ | 12 | 105-106 | 105-106 | 50-55 | 03-06 | 56-62 | yellow | — | — |
| 2,6-(CH ₃) ₂ | 13 | 92-93 | 85-88 | 52-56 | 04-07 | 55-61 | tan | — | — |
| 2,5-(CH ₃) ₂ | — | — | 133-135 | 50-55 | 03-07 | 55-60 | yellow | — | — |
| 2,3,5-(CH ₃) ₃ | 14 | 112 | 109-110 | 48-54 | 01-05 | 54-58 | yellow | — | — |
| 2,3,5,6-(CH ₃) ₄ | 15 | 202-203 | 207-208 | 37-41 | 04-07 | — | yellow | — | — |

| | | | | | | | | | |
|---|-----------|-----------|---------|-------|-------|-------|--------|------|-------------------|
| 5,7-(CH ₃) ₂ -6-(OH) | 16 | 92.5-93.5 | 98-99 | 45-51 | 24-31 | 55-64 | yellow | blue | grey |
| 5,8-(CH ₃) ₂ -6-(OH) ^b | 16 | 77-78 | 91-92 | 27-34 | 14-21 | 54-63 | yellow | blue | grey |
| 5,7,8-(CH ₃) ₃ -6-(OH) | 17 | 94-94.5 | 93-94 | 45-55 | 20-26 | 68-73 | yellow | blue | grey |
| 5,7,8-(CH ₃) ₃ -6-(CO ₂ CH ₃) | — | — | 92-93 | 64-68 | 22-28 | 64-75 | yellow | — | — |
| <i>Dichromans</i> | | | | | | | | | |
| Unsubstituted (I) | — | — | 159-161 | 65-71 | 41-48 | 72-79 | tan | blue | — |
| o-(CH ₃) ₂ (II) ^b | 18, 16 | 101-102 | 97-100 | 75-79 | 63-70 | 74-80 | yellow | blue | — |
| p-(CH ₃) ₂ (III) | 16 | 193-196 | 191-193 | 74-78 | 64-71 | 77-84 | yellow | blue | — |
| <i>Oxidation products</i> | | | | | | | | | |
| Red (IV) ^c | 19 | 109-110 | — | 22-27 | 00-04 | 54-59 | brown | blue | — |
| Purple (V) ^c | 16 | 142-143.5 | — | 00-04 | 00-03 | 05-08 | brown | blue | grey |
| Quinone (VI) ^c | 20 | 62 | — | 09-15 | 00-03 | 52-57 | tan | blue | — |
| Yellow dimer (VII) ^b | 3, 21, 22 | 126-127 | 77-79 | 63-68 | 17-25 | 72-76 | green | blue | grey ^d |
| Dihydroxy dimer (VIII) | 21 | — | 185-188 | 45-51 | 12-17 | 66-75 | yellow | blue | grey |
| Trimer (IX) | 3 | — | 227-228 | 73-78 | 28-35 | 77-83 | brown | blue | — |

^a All of the diacetates, hydroxychromans, and dichromans were prepared from their respective hydroquinones.

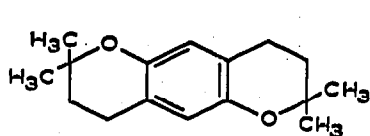
^b The NMR spectrum and elementary analysis were consistent with the structure proposed

^c The IR spectra of these compounds were identical with authentic samples.

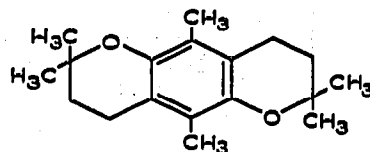
^d After one hour.

Preparation of 2,2,7,7-tetramethyl-3,8-dihydrobenzo[1,2-b:4,5-b']dipyran (I)

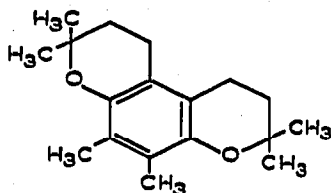
This compound was prepared in good yield by treating hydroquinone with excess isoprene in refluxing acetic acid containing zinc chloride as described previously^{18,19}. After several recrystallizations from alcohol-water mixtures and finally vacuum sublimation, the compound melted at 159–161° and showed no OH stretching bands in the infrared. The NMR spectra showed a singlet at 8.75 τ ; two triplets at 8.32 τ and 7.35 τ and a singlet at 3.70 τ which stood in the area ratio of 6:2: 2:1 respectively.



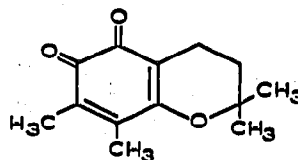
I



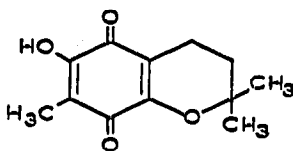
II



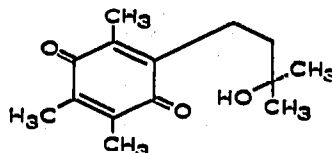
III



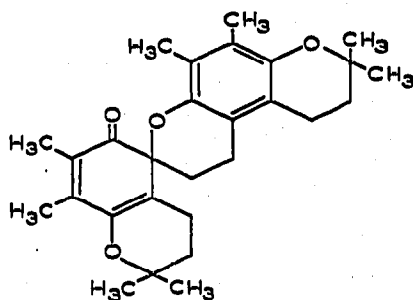
IV



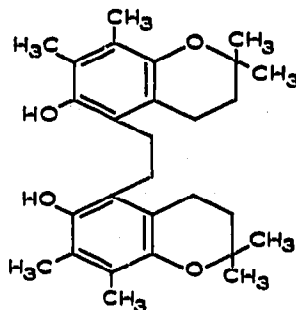
V



VI



VII



VIII

Trimer of unknown structure³
IX

A comparison of this NMR spectra with that of 3,3,5,6,8,8-hexamethyl-2,9-dihydrobenzo[1,2-b:4,3-b']dipyran (III), which showed triplets at 8.27 τ and 7.48 τ tends to support the assignment of structure I to this compound as indicated by the up field shift of its more shielded methylenic protons.

Analysis. Calculated for C₁₆H₂₂O₂: C, 78.0; H, 8.94. Found: C, 78.0; H, 9.09.

Preparation of 2,5-dimethylhydroquinone diacetate

The diacetate of 2,5-dimethylhydroquinone was prepared by refluxing the hydroquinone in excess acetic anhydride-pyridine mixture for 1 h. The mixture was poured into cold water and the white crystalline compound obtained in nearly quantitative yield. It was recrystallized once from ethanol and then sublimed in a vacuum, m.p., 133-135°.

Analysis. Calculated for $C_{12}H_{14}O_4$: C, 64.9; H, 6.31. Found: C, 65.5; H, 6.82.

Preparation of 2,2,5,7,8-pentamethyl-6-acetoxychroman

This compound was prepared exactly as described above for the 2,5-dimethylhydroquinone diacetate except that the compound was not sublimed. The recrystallized material, shining plates, was dried in a vacuum at room temperature, m.p., 92-93°.

Analysis. Calculated for $C_{16}H_{22}O_3$: C, 73.3; H, 8.39. Found: C, 72.8; H, 8.46.

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SUMMARY

Characterization of a number of phenolic compounds related to the tocopherols and their oxidation products by the use of thin-layer chromatography on silica gel is described.

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