*Phytochemistry*, Vol. 21, No. 3, pp. 794–795, 1982.
Printed in Great Britain.

0031-9422/82/030794-02$03.00/0


# CHROMATOGRAPHIC AND SPECTRAL CHARACTERIZATION OF THE METHYLCYANIDINS PRODUCED BY DEMETHYLATION

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(*Received* 8 *April* 1981)

**Key Word Index**—Synthetic flavylium salts; controlled demethylation; methylcyanidins; chromatographic and spectral characterization.

**Abstract**—The synthetic methoxyflavylium salts in the cyanidin series were demethylated in pyridinium chloride under nitrogen. The order of demethylation of the methoxyl groups was 3, followed by 7 and 5 and then 3′ and 4′. Twelve methyl ethers, including paeonidin, elodenidin (5-methylcyanidin) and rosinidin were produced by demethylation. Chromatographic and spectral characteristics of these methylcyanidins are described.

## INTRODUCTION

Controlled demethylation is useful for the identification of methylanthocyanidins. Harborne first applied the procedure to confirm the structure of capensinidin (5-methylmalvidin) [1]. The authors detected elodenidin (5-methyl cyanidin) from leaves of *Egeria densa* (Planch.) St. John [2]. In order to compare this pigment with 7-methylcyanidin, rosinidin (7-methylpaeonidin) was demethylated but no 7-methylcyanidin was detectable. Abe *et al.* [3] previously synthesized six methoxyflavylium salts as model compounds of cyanin in which various hydroxyl groups were substituted by methylation. To make clear the course of demethylation, these synthetic pigments were used (Table 1). In this report, the chromatographic and spectral characteristics of the 12 methylcyanidins produced during demethylation are described.

## RESULTS AND DISCUSSION

When 3,5-dimethylcyanidin was demethylated in pyridinium chloride, three pigments were detected. At first the original pigment and elodenidin (after 5 min), then elodenidin and cyanidin (after 10 min) and lastly only cyanidin (after 20 min) were detected. In the case of the demethylation of 3,5,7- and 3,5,3′-trimethylcyanidins, the methylcyanidins produced were identified by comparison with each other and with authentic pigments. The 5,7-di-, 5,3′-di-, 5-mono-, 7-mono-, 3′-monomethyl ethers and cyanidin were produced (Table 1). Generally speaking, the 3-methyl group is removed at first, then A-ring methyl groups are more easily removed than those in the B-ring. Finally, within the A-ring, the 5-methyl group is more resistant than the 7-methyl, and in the B-ring, the 4′-methyl is more resistant than the 3′-methyl.

Twelve methylcyanidins, mono-, di-, tri- and

Table 1. The course of demethylation of cyanidin methyl ethers

| Sample | Methylcyanidins produced by demethylation | | | |
|---|---|---|---|---|
| | TetraOMe | TriOMe | DiOMe | MonoOMe |
| 3,5-DiOMe | | | | 5- |
| 3,5,7-TriOMe | | | 5,7- | *5-*<br>7- |
| 3,5,3′-TriOMe | | | 5,3′- | 5-<br>*3′-* |
| 3,5,7,3′-TetraOMe | | 5,7,3′- | *5,3′-*<br>7,3′- | 5-<br>*3′-* |
| 3,5,3′,4′-TetraOMe | | 5,3′,4′- | 5,3′-<br>*3′,4′-* | 5-<br>*4′-* |
| 3,5,7,3′,4′-PentaOMe | 5,7,3′,4′- | *5,3′,4′-*<br>7,3′,4′- | 5,3′-<br>*3′,4′-* | 5-<br>*4′-* |

Italic numbers indicate major pigments formed.

Table 2. $R_f$ values and spectral maxima of the methylcyanidins

| Pigments | $R_f$ (100×) Formic* | $R_f$ (100×) Forestal† | $\lambda_{vis\,max}$ (nm) 1% MeOH–HCl | $\frac{E_{440}}{E_{max}}$ (%) | Fluorescent colour short wave |
|---|---|---|---|---|---|
| Cyanidin | 18 | 45 | 539 | 24 | Red |
| 3′-OMe (Pn) | 27 | 60 | 538 | 30 | Dull orange |
| 7-OMe | 28 | 61 | 536 | 16 | Red |
| 4′-OMe | 30 | 64 | 537 | 22 | Dull orange |
| 5-OMe (Ed) | 34 | 67 | 539 | 21 | Orange |
| 7,3′-DiOMe (Rs) | 37 | 77 | 533 | 17 | Light orange |
| 3′,4′-DiOMe | 39 | 77 | 534 | 26 | Dull orange |
| 5,3′-DiOMe | 42 | 79 | 537 | 27 | Orange |
| 5,7-DiOMe | 46 | 79 | 533 | 19 | Dull orange |
| 7,3′,4′-TriOMe | 47 | 87 | 530 | 14 | Light orange |
| 5,7,3′-TriOMe | 52 | 91 | 531 | 10 | Orange |
| 5,3′,4′-TriOMe | 54 | 91 | 532 | 20 | Orange |
| 5,7,3′,4′-TetraOMe | 63 | 98 | 528 | 48 | Orange |

*Formic, formic acid–conc. HCl–$H_2O$ (5:2:3).
†Forestal, acetic acid–conc. HCl–$H_2O$ (30:3:10).

tetramethyl ethers, were detected as the intermediates. Their chromatographic and spectral characteristics are summarized in Table 2. The pigments are arranged in order of their $R_f$ values in the Formic solvent, because they are best separated with this solvent system on TLC.

As the number of methyl groups increases, the absorption maxima in the visible region tend to occur at shorter wavelengths. The size of the hypsochromic shift in each position is additive. At about 440 nm, some pigments have a weak peak and this may be characteristic, so that the $E_{440}/E_{vis\,max}$ values are useful for spectral characterization.

The absorption spectra of elodenidin and 7-methylcyanidin are also shown in Table 2. Elodenidin showed the $\lambda_{vis\,max}$ at 539 nm and 7-methylcyanidin at 536 nm. The $E_{440}/E_{vis\,max}$ values are also different. $R_f$ values are 0.34 and 0.28 with Formic, 0.67 and 0.61 with Forestal, respectively. The fluorescence of the former was orange and the latter red. Thus, the two pigments are clearly distinguishable.

As Harborne has mentioned [4], 5-Me derivatives have an orange fluorescence in UV light on TLC. A weak fluorescence is also found in 3′- or 4′-methylcyanidin. 5,3′-Dimethyl ethers, such as 5,3′-di-, 5,7,3′-tri-, 5,3′,4′-tri- and 5,7,3′,4′-tetramethylcyanidin, fluoresce vividly. The fluorescence in UV light may be one of the most valuable criteria for the identification of methylcyanidins.

Theoretically there are 15 methylcyanidins, because all natural anthocyanidins contain a free hydroxyl group in the 3-position. We have obtained 12 methylcyanidins including three natural pigments, paeonidin, elodenidin and rosinidin. Three other pigments, 5,4′-di, 7,4′-di- and 5,7,4′-trimethylcyanidin may be obtained by the demethylation of the 3,5,7,4′-tetramethyl or 5,7,4′-trimethyl ethers. Controlled demethylation is convenient and useful for determining the structure of methylanthocyanidins including the aglycones of anthocyanidin glycosides following methylation [5].

## EXPERIMENTAL

*Synthetic pigments.* See refs. [2] and [3].

*Demethylation procedure.* Pyridinium chloride (1–2 g) and the pigment (1–2 mg) were heated at 160° under $N_2$ and samples were taken after 5, 10, 20 and 30 min. Demethylated pigments were dissolved in 1% HCl, and extracted with a small amount of 1% *iso*-amylalcohol–HCl. The pigments were spotted on TLC and developed with Formic and Forestal.

*Isolation and determination of methylcyanidins.* Methylcyanidins were separated on TLC and re-extracted with 1% MeOH–HCl. Determination of these isolated methylcyanidins were established by $R_f$ values, the absorption spectra and by further demethylation. They were compared with authentic samples of cyanidin, paeonidin, elodenidin, rosinidin and synthetic 4′-methylcyanidin.

*Acknowledgements*—We are grateful to Professor J. B. Harborne for providing rosinidin and also to Dr. K. Yoshitama, Biological Institute, Shinshu University, for providing 4′-methylcyanidin. Thanks are due to Professor K. Hayashi, Research Institute of Evolutionary Biology, for his helpful suggestion and encouragement.

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