Ageratochromene, a Heterocyclic Compound from the Essential Oils of some *Ageratum* Species

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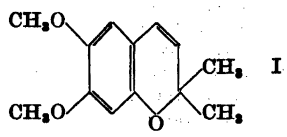
During researches carried out in this laboratory on acetylenic compounds from *Compositae*, some aromatic substances have been isolated. From the essential oils of *Ageratum mexicanum* Sims. and *A. conyzoides* L. a crystalline compound (I) $C_{13}H_{16}O_3$, for which we propose the name ageratochromene, was obtained. It had m.p. 47.5° (corr.) and was optically inactive (Found: C 70.8; H 7.35. Calc. for $C_{13}H_{16}O_3$: C 70.9; H 7.35), λ_{max} 2 800, 3 230 Å (ε_{max} 5 500 and 9 300, respectively). The infra-red spectrum revealed the aromatic character, the presence of methyl and ether groups and the absence of any carbonyl or hydroxyl functions. Catalytic hydrogenation furnished a dihydrocompound (II), m.p. 60° (corr.), λ_{max} 2 930 Å (ε_{max} 6 400). The U. V.-spectra of (I) and (II) indicated the presence of a benzene nucleus conjugated with one double bond; it then follows that (I) must be bicyclic. In contrast to (I) the dihydrocompound (II) gave a satisfactory result in the methoxyl determination. (Found: MeO 28.5. Calc. for $C_{13}H_{18}O_3$ (2 MeO): MeO 27.9). Oxidation of (I) with OsO_4 gave a glycol (III), m. p. 129.5° (corr.) (Found: C 61.35; H 7.1. Calc. for $C_{13}H_{18}O_5$: C 61.4; H 7.15). λ_{max} 2 925 (ε_{max} 4 300). Chromium trioxide oxidation of (III) gave a compound (IV) $C_{13}H_{16}O_5$, m. p. 116–118.5° (corr.). (Found: C 61.2; H 6.7 $C_{13}H_{16}O_5$ requires C 61.9; H 6.4). According to a strong I.R.-peak at 1 682 cm^{-1} (IV) contains a carbonyl group conjugated with an aromatic ring. The U.V.-spectrum, λ_{max} 2 420, 2 745, 3 375, 3 483 (ε_{max} 55 200, 13 500, 7 600 and 6 500, respectively) confirms this. Oxidation of (III) with lead tetra-acetate afforded another substance (V) $C_{13}H_{16}O_5$, m. p. 101.5–102° (corr.) (Found: 61.5; H 6.5; MeO 25.1. $C_{13}H_{16}O_5$ requires C 61.9; H 6.4; MeO 24.6), λ_{max} 2 730, 3 160 Å (ε_{max} 15 200 and 7 700, respectively). According to the U.V.-spectrum (V) should have one carbonyl group conjugated with the benzene ring, and this was confirmed by an infra-red maximum at 1 676 cm^{-1}. The infra-red spectrum also showed a strong peak at 1 740 cm^{-1} indicating another carbonyl function coinciding with the standard value for an isolated ester group. From this indication (I) was supposed to be a 1 *H*-2-benzopyran derivative and should give a dimethoxyphthalic acid when submitted to a further oxidative degradation. All experiments in this direction were unsuccessful.

Control measurements of phenoxyacetone gave an infra-red maximum at 1 736 cm^{-1}, $\Delta\nu$ = 16 cm^{-1}. For phenoxyacetaldehyde this maximum was shifted to 1 745 cm^{-1}, $\Delta\nu$ = 13 cm^{-1}. This indicated that the 1 740 cm^{-1} maximum of (V) might belong to an isolated carbonyl group and that it was displaced from its ordinary position by the presence of an α-phenoxy-residue, compare [1].

(V) could be oxidized further with potassium permanganate to a monocarboxylic acid (VI) $C_{13}H_{16}O_6$, m. p. 118–119° (corr.) (Found: C 58.0; H 6.0. Calc. for $C_{13}H_{16}O_6$: C 58.2; H 6.0), λ_{max} (in ethanol) 2 745, 3 320 (ε_{max} 8 500 and 5 400, respectively) and a dicarboxylic acid (VII) $C_{13}H_{16}O_7$, m. p. 157–159.5° (corr.) (Found: C 54.6; H 5.45. Calc. for $C_{13}H_{16}O_7$: C 54.9; H 5.65), λ_{max} (in ethanol) 2 570, 3 000 (ε_{max} 12 900 and 7 100 respectively). The double bond conjugated with the benzene ring therefore would be of the type $-CH=CH-$. As the glycol (III) gives a monoacetate (VIII), a monoketone (IV) and a monocarboxylic acid (VI), the carbon atom next to the double bond might be substituted in a manner as to give an appreciable steric hindrance. The infra-red spectra of (I) and all its derivatives showed a splitting of the methyl band into two well resolved maxima at 1 383 and 1 363 cm^{-1}. A *gem.*dimethyl group was probable, and (I) might be supposed to be a dimethoxy-2:2-dimethyl-chromene. The infra-red spectra of (I) and its derivatives further indicated a 1,2,3,5- or a 1,2,4,5-substituted benzene compound. The 5:7-dimethoxy-2:2-dimethyl-chroman (IX) had been synthesized by Alexander Robertson *et al.*[2] and (IX) was stated to be a liquid. A resynthesis confirmed this. The infra-red spectrum of (IX) showed many similarities with that of (II), but also the non-identity of the two substances. 6:7-Dimethoxy-2:2-dimethyl-chroman (X) was then synthesized following an analogous procedure, and (X) turned out to be iden-

tical with (II) according to m. p., mixed m.p. and I.R.-spectrum. Ageratochromene is therefore 6:7-dimethoxy-2:2-dimethyl-chromene.



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The 2:2-dimethyl-chromene skeleton has been found in other natural compounds *e. g.* deguelin, toxicarol, xanthoxyletin, xanthyletin [3] and evodionol [4]. So far ageratochromene is the first compound of this class isolated from a plant belonging to the *Compositae*. Ageratochromene has the same type of benzene substitution as ayapin [5] (6:7-methylenedioxycoumarin) isolated from the closely related plant *Eupatorium ayapana* Vent. (*Ageratum* and *Eupatorium* both belong to subtribus Ageratinae, tribus Eupatorieae), scoparone [6] (6:7-dimethoxy-coumarin) isolated, *e. g.*, from the composite plants *Artemisia scoparia* and *A. capillaris*, and cichoriin [7] (6-hydroxy-7-glucosido-coumarin) isolated from the flowers of chicory.

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Effect of Cobalt and Iron on Riboflavin Production by *Candida guilliermondia*

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The yeast *Candida guilliermondia*, which is capable of outstanding synthesis of riboflavin, was first investigated by Burkholder [1]. Tanner *et al.*[2] found that iron, if present in the medium at a concentration of 100 μg/l, sharply reduced the riboflavin production of *C. guilliermondia*. The optimum iron concentration, according to these authors, is 5—10 μg/l. Earlier Arzberger [3] had shown that iron and cobalt, at a concentration of 3.2 mg/l, reduced the riboflavin production of *Clostridium acetobutylicum*, while zinc, copper, and lead had no influence.

In the present work it is shown that cobalt in a concentration of 10^{-4} M (5.9 mg/l) considerably enhances the riboflavin production of *C. guilliermondia* and shifts the optimum iron concentration to about 10^{-5} M (560 μg/l).

Methods. The *C. guilliermondia* strain used in this work was originally sent to the Centraalbureau voor Schimmelcultures, Baarn, Holland, by P. Burkholder, as synthesizing riboflavin. For the present work it was obtained from Mrs. June Robson, Isotope Division, A.E.R.E., Harwell, England, to whom I wish to express my thanks. The yeast was cultivated in the following medium: glucose 30 g, $(NH_4)_2HPO_4$ 3 g, KH_2PO_4 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, biotin 5 μg, and water 1 000 ml. The pH was adjusted to 4.5. All chemicals used were of analytical grade and the water was distilled twice in a quartz glass apparatus. Iron was not removed from the medium nor was the iron content of it determined. The yeast was cultivated at 30° C with powerful aeration. The riboflavin produced was determined by direct spectrophotometry of the centrifuged medium.

Results. *C. guilliermondia* was grown in the nutrient medium with different amounts of cobalt sulphate added. Samples were taken after 1, 5, 24, and 30 hours. After 1 and 5 hours no riboflavin could be detected in the medium. The amounts of