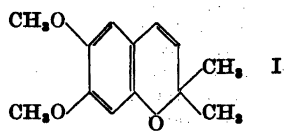
tical with (II) according to m. p., mixed m.p. and I.R.-spectrum. Ageratochromene is therefore 6:7-dimethoxy-2:2-dimethyl-chromene.



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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

riboflavin produced in 24 and 30 hours can be seen from Fig. 1. The curves show a very distinct maximum for riboflavin production at a cobalt concentration of 10^{-4} M. Even at a concentration of 10^{-2} M cobalt inhibits the growth of the yeast and at 10^{-3} M does so to some extent, but at 10^{-4} M does not affect growth.

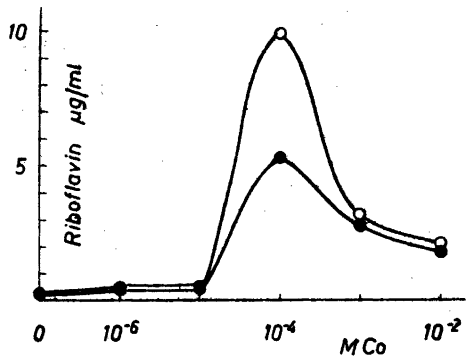

Fig. 1. Effect of cobalt on riboflavin production by C. guilliermondia. *Riboflavin estimated after 24 hours' (●) and 30 hours' (○) growth.*

Some other divalent cations and molybdate were tested at concentrations of 10^{-4} M. The only one of these, apart from cobalt, causing a sligth increase in the riboflavin production was zinc (Table 1).

Table 1. Effect of some ions on riboflavin production at concentrations of 10^{-4} M. Riboflavin estimated after 20 hours' growth.

Ion added	Riboflavin in the medium µg/ml
None	2.6
Co++	13.6
Mn++	0.3
Zn++	4.7
Cu++	0.0
MoO_4^{--}	0.0

In another experiment the yeast was grown in a medium containing 2×10^{-4} M cobalt and with different amounts of iron added (Table 2). Controls with no cobalt showed that these additions of iron completely inhibited the production of riboflavin. In the cobalt-containing medium the highest riboflavin production was obtained when 10^{-5} M of iron was added. The addition of 2×10^{-4} M of cobalt thus shifts the optimum iron concentration from about 10^{-7} M to 10^{-5} M. It seems likely that there is competition between cobalt and iron and that the action of cobalt on riboflavin production is due, at least in part, to this competition.

Table 2. Effect of cobalt and iron on riboflavin production. Riboflavin estimated after 30 hours' growth.

Cobalt added M	Iron added M	Riboflavin in the medium mg/g yeast (dry matter)
0	0	1.5
0	10^{-5}	0.0
0	10^{-3}	0.0
2×10^{-4}	0	2.7
2×10^{-4}	10^{-5}	3.9
2×10^{-4}	10^{-3}	1.0

Note. Hickey (*U. S. Patent* 2 667 445 (1954); Ref. in *Chem. Abstracts* 48 (1954) 6 084) has found that 10 mg/l of cobalt increases the riboflavin production of *Ashbya gossypii* by about 60 %, while 50 mg/l of cobalt reduces it by about 70 %.

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