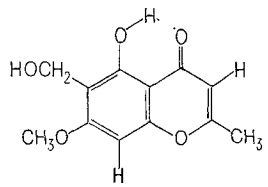
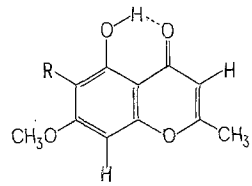
Isolation of 6-hydroxymethyl-eugenin from *Chaetomium minutum*

From culture filtrates of the chaetocin-producing fungus *Chaetomium minutum*[1], we have isolated a minor metabolite, m.p. 199–202°, which analyses as $C_{12}H_{12}O_5$ ($M^+ = 236$). On the basis of chemical and spectroscopic evidence summarized below, we propose structure **1** for the new compound.

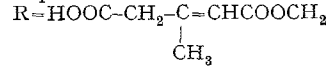




1 6-hydroxymethyl-eugenin **2** $R = CH_3COOCH_2-$

3 eugenitin $R = CH_3-$

4 lepraric acid



5 eugenin $R = H$

The NMR-spectrum in $(CD_3)_2SO$ shows signals for a vinylic methyl at $\delta = 2.38$ ppm and a methoxyl group at 3.88 ppm. The singlet at 4.46 ppm is consistent with the presence of a hydroxymethyl group (ν_{max} 3420 cm^{-1}), which on acetylation (**2**, m.p. 165–166° $M^+ = 278$) is shifted downfield to 5.02 ppm (in $(CD_3)_2SO$). The second hydroxyl ($\delta = 13.05$ ppm) is strongly hydrogen-bonded and cannot be acetylated with Ac_2O in pyridine. The IR-absorptions at 1660, 1625 and 1575 cm^{-1} (KBr) are characteristic of a chelated chromone[2,3]. The UV-spectrum (in dioxane) with maxima at 234, 251, 257, 288 and 315 nm (infl) (log ε = 4.29, 4.26, 4.26, 3.87) closely resembles those of eugenitin (**3**)[4] and lepraric acid (**4**)[5,6]. The identity of the chromophore system was established by hydrogenolytic (Pd/C 10%, in dioxane) conversion of **1** into eugenitin (**3**).

The chemical correlation with eugenitin (**3**) does not determine the point of attachment of the hydroxymethyl group. This problem was solved by NMDR. Decoupling of the methyl group at 2.38 ppm (**1**, in $(CD_3)_2SO$) causes a significant enhancement of the signal at 6.16 ppm ($J < 0.5$ Hz), whereas no such effect was found with the methylene group. This clearly indicates the vinylic position of the respective proton. Irradiation of the methoxy group and a nuclear Overhauser effect[7] of approx. 20% for the proton at 6.55 ppm, which is in good agreement with the aromatic position as assigned in **1**, was observed.

It is interesting to note that the same chromone system is present in eugenin (**5**) and eugenitin (**3**), found in the wildgrowing clove *Eugenia caryophyllata*[4,8], in lepraric acid (**4**) a constituent of various lichen species[5,6], and in the mould metabolite 6-hydroxy-methyl-eugenin (**1**). The latter is devoid of antimicrobial activity[9].

Zusammenfassung. 6-Hydroxymethyl-eugenin (**1**) wurde als Nebenprodukt von Chaetocin aus dem Pilzstamm *Chaetomium minutum* isoliert. Eugenin und Eugenitin, die Inhaltsstoffe der Nelke *Eugenia caryophyllata*, Learia-säure aus verschiedenen Flechten, sowie der Pilzmetabolit **1** unterscheiden sich nur in Stellung 6 des Chromon-gerüstes.

D. HAUSER and THERESE ZARDIN

Pharmaceutical Chemical Laboratories, SANDOZ Ltd., CH-4002 Basel (Switzerland), 15 February 1972.

1 D. HAUSER, H. P. WEBER and H. P. SIGG, Helv. chim. Acta *53*, 1061 (1970).
2 K. NAKANISHI, *Infrared Absorption Spectroscopy* (Holden Days, San Francisco 1972), p. 52.
3 C. N. RAO, *Chemical Appl of Infrared Spectroscopy* (Academic Press, New York 1963), p. 457.
4 H. SCHMID, Helv. chim. Acta *32*, 813 (1949).
5 K. SOVIAR, O. MOTL, Z. SAMEK and J. SMOLIKOVA, Acta Fac. Pharm., Bratisl. *17*, 39 (1969).
6 D. J. ABERHART, K. H. OVERTON and S. HUNEK, J. chem. Soc. (C) *1969*, 704.
7 For a similar case see: R. A. BELL and J. K. SAUNDERS, Can. J. Chem. *48*, 1114 (1970).
8 Th. M. MEIJER, Rec. Trav. chim. Pays-Bas *65*, 843 (1946).
9 Acknowledgments: We thank Prof. H. SCHIMD, University of Zürich, for a sample of eugenitin. The technical assistance of Miss S. ROMINGER is gratefully acknowledged.

New Anti-Malarial Agent with Potential Repository Effect

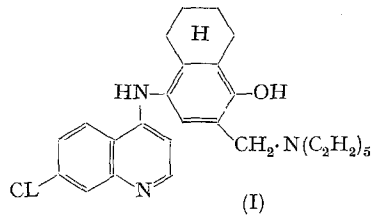
Drugs of the aminoquinoline type have been known for their activity against the erythrocytic stages of the malaria parasite in humans[1]. Another group of compounds which might acquire their biological effectiveness as antagonists of Vitamin K, and are of considerable antimalarial activity, consists of 2-hydroxy, 3-alkyl, 1,4-naphthoquinone and related derivatives[2].

Thus, the development of new agents that would structurally include systems related to naphthoquinones along with the N-heterocyclics could be useful as antimalarial agents, since the resistance of the parasite to nitrogen-heterocyclics should not imply resistance to compounds of the naphthoquinone type as both are reacting through different mechanisms. These considerations suggested to us new substituted naphtholic compounds and their reduced tetrahydro- derivatives as side chains attached to 4-aminoquinolines and 9-amino-acridines systems. The chemistry of these compounds with the different approaches for their syntheses had been fully described[3]. Related compounds of the type 4-amino, 1-naphthol and its reduced tetrahydro-form, 4-amino, 1-hydroxy, 5,6,7,8-tetrahydronaphthalene, could readily undergo chemical oxidation to give the corresponding naphthoquinoid structure[4]. As representative for this group of compound-*s*, 4-(7-chloro-4, aminoquinolyl)-2, diethylaminomethyl-5, 6,7,8-tetrahydro-1-naphthol (I) was selected for testing for antimalarial activity.

The compound dissolves readily in water and is colourless. These properties provide the advantages for possible

1 World Health Organ. Tech. Rept. Ser. *226* (1961).
2 L. FIESER and A. RICHARDSON, J. Am. chem. Soc. *70*, 3156 (1948).
3 I. NABIH, M. NASR and A. BADAWI, J. pharm. Sci., to be published (1972).
4 R. ARNOLD and H. ZAUG, J. Am. chem. Soc. *63*, 1317 (1941).

practical applications without facing the problems of skin colouring and insolubility. Chemically, compound I could be prepared through condensation of both moieties, 2-diethylaminomethyl-4-amino-5, 6, 7, 8-tetrahydro-1-naphthol and 4, 7-dichloroquinoline. The former is accessible through a Mannich reaction (formaldehyde/diethylamine) with 4-acetamino-5, 6, 7, 8-tetrahydro-1-naphthol followed by acid hydrolysis.



(I)

Biological testing was based on the response to the product by *Plasmodium berghei* in mice. For this purpose mice of NMRI-SPF-Hann. strain (males) were used of weights 18–22 g. Infection was induced in the mice groups through i.p. inoculation of heparinized infected blood diluted with physiological saline solution. Inoculum for each mouse was 0.3 ml with ca. 10^7 infected erythrocytes with K173 strain of *Plasmodium berghei*.

Oral treatment with the compound in water solution followed 2 h after infection. The various doses in the different groups of infected mice were given daily once for 4 consecutive days. Regular blood microscopical examinations were carried out (Giemsa staining), 3 days after infection for a period of 4 weeks post-infection. Criteria for a given dose to be curative: when no parasites could be microscopically detected in the blood samples withdrawn from the treated animals. The same animals were submitted to further reinfection and if the new infection is established as in controls, then the animals that had been initially treated with these doses were considered to be cured and the given doses as curative. The re-exposure to infection was to secure the rationalization of the absence of the parasites in the treated animals as due to treatment and not due to preimmunity factors. In these studies dosages range of 5 mg/kg body weight through to 100 mg/kg were found to be curative. Lower doses of 1 mg/kg were inactive and of 2.5 mg/kg the activity was poor.

Further the compound I was tested for its repository effect. For this purpose single oral doses of 100 mg/kg body weight and 250 mg/kg were given to mice 1 and 2 weeks before infection with Schizontes bearing erythrocytes. This oral application was shown to be inactive to protect the animals against infection. On the other hand, s.c. applications of the same single doses, 100 mg and 250 mg/kg body weight could provide complete protection against infection with the parasite for a period of at least 2 weeks. Inspite of the exposure to massive infection, no parasitemae could be observed for a period of 4 weeks post infection exposure.

In addition, compound I was evaluated for its antimalarial activity in another group of experiments. This was based on comparison of response to compound by *Plasmodium berghei* KG173 malaria in mice as expressed in mean survival times and the mean survival times of untreated controls[5]. A single dose at the desired level is given 72 h after mice infection with *P. berghei*. To consider the tested compound as active, a minimum survival time of 13 days is required. If the treated mice lived 60 days or more, the compound is considered as curative. In these experiments, doses of 5 mg/kg body weight gave an increase in survival days of 3.7. Doses of 10 mg caused increase in the survival days by 12.3. Cures were affected (5/5) at doses of 40 through 350 mg/kg body weight. At 20 mg/kg cure rate was 4/5.

These biological findings show that the compound I possesses both curative and prophylactic effect against malaria infection in mice. This may offer a lead for future syntheses of compounds bearing structural features of biologically active groupings of antimalarials as for the substituted naphthoquinones and nitrogen-heterocyclics[6].

Zusammenfassung. Ein potentielles Malariamittel, welches Strukturelemente wirksamer Heterocyclen mit denjenigen der Naphtochinone vereint, wurde in orientierenden Versuchen als wirksam gefunden.

I. NABIH[7]

National Research Center, Dokki, Cairo (Egypt), 13 March 1972.

[5] T. OSDENE, P. RUSSEL and L. RANE, J. med. Chem. *10*, 431 (1967).

[6] Acknowledgment. The author wishes to express his thanks to Prof. Dr. R. GÖNNERT, Dr. HABERKORN and the group of Farbenfabriken Bayer AG., Wuppertal-Elberfeld, W. Germany, for their distinguished help and cooperation for the compound tests. Also, his gratitude to Prof. Dr. J. BURCKHALTER, University of Michigan, and the Walter Reed Institute of Research, USA for their cooperation for the biological evaluation of the product.

[7] Present address. Medicinska Nobel Institutet, Biokem. Avdl., Laboratory for Enzyme Research, Solnavägen 1, Stockholm 60 (Sweden).

Behaviour of a Guanidine-Dependent Strain of Poliovirus 1 in Sucrose Density and pH Gradients

Results of recent researches indicate that guanidine invalidates the functions of newly-formed Enterovirus proteins[1,2]. The guanidine marker is thought to be a part of the nucleotide sequence of viral RNA which codes for protein synthesis[3]. The researches reported here have been carried out to establish if a state of guanidine dependence is accompanied by modifications in the density and in the isoelectric point of the viral particle.

Materials and methods. Researches have been carried out by using a concentrated, Genetron-treated suspension in Hank's BSS of both a Brunenders strain of poliovirus 1 and its guanidine dependent variant G 200, which requires 200 µg/ml of guanidine HCl for optimal growth.

To study viral density, 0.5 ml of viral suspension were layered on the top of a preformed 4.5 ml sucrose gradient (42–30%) in *Tris* mg buffer (0.05 *M Tris*, 0.025 *M* KCl, 0.005 *M* $MgCl_2$ mg; pH 7.45) in 5 ml tubes of cellulose nitrate, and centrifuged in Spinco (rotor SW 50) at 35,000 rpm × 100 min at + 4 °C. Fractions were collected from the bottom of the tubes. Plaque forming units of either viruses were titrated according to the DULBECCO and VOGT technique[4] taking advantage of the fact that 60 µg/ml of