Green tea to cure bad breath. The role of polyphenols clarified

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Abstract: (-)-Epigallocatechin gallate (EGCg), the main polyphenol in green tea, showed deodorizing activity against CH_3SH. The chemical reaction between EGCg and CH_3SH was investigated. The non-volatile reaction products were purified and identified. They were found to be EGCg derivatives carrying methylthio and/or a methylsulphinyl groups on the B ring.

The effect of green tea on halitosis has been well known from early times in Japan and we have previously reported the deodorizing activity of tea catechins, the main components of green tea, against methanethiol (CH_3SH), the main source of halitosis (Ui *et al.*, 1991). Other natural substances possessing high deodorizing activities against CH_3SH have been found in some plants, most of them being polyphenols and phenolic derivatives (Yasuda and Ui, 1992; Nakatani *et al.*, 1989; Miura *et al.*, 1989; Kita *et al.*, 1990). The deodorizing mechanism of polyphenols against CH_3SH is considered to involve the formation of hydrogen bonds between phenolic hydroxyl groups and the thiol group, a clathration, physical association such as adsorption, and an addition reaction, although each has not yet been clarified (Kita *et al.*, 1990).

Amongst tea catechins, EGCg and (-)-epigallocatechin (EGC), both having three hydroxyl groups at the 3', 4' and 5' position of the B ring, showed high deodorizing activity against CH_3SH. The activity order of tea catechins measured by monitoring the loss of CH_3SH by gas chromatography is EGCg >EGC > (-)-epicatechin gallate (ECg) > (-)-epicatechin (EC) (Ui *et al.*, 1991) . The anti-oxidative activity per mole of these catechins follows the same order (Matsuzaki and Hara, 1985), the order of deodorizing activity generally matching that of anti-oxidative activity.

To better understand the deodorizing mechanism of tea catechins, we have limited our present study to the deodorizing reactions of EGCg, and we tried to find whether EGCg would chemically react with CH_3SH to give new products. EGCg was reacted in a sealed tube at 37°C with an excess of methanethiol sodium salt (CH_3SNa) in a phosphate buffer of pH 7.5 (corresponding to the average pH of stimulated saliva) and the reaction mixture was analysed by HPLC. The peak of EGCg decreased with increasing time, and new peaks appeared at retention times of 13.3, 15.6 and 17.8 min. The evolution of their relative areas with time suggested that they could correspond to successive intermediates (Fig. 1) (Yasuda and Arakawa, 1995).

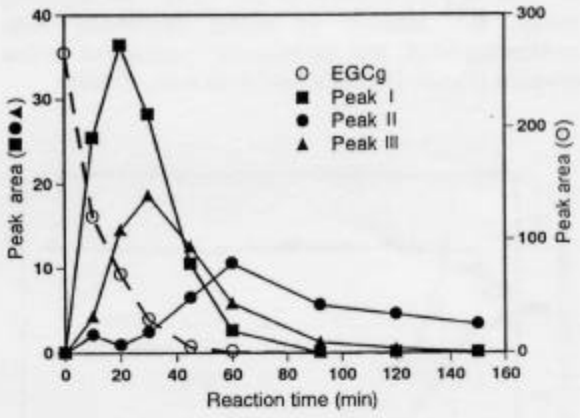

Figure 1. Time-Course of the Reaction between EGCg and CH_3SNa as measured by the HPLC peak area at 280 nm. Both EGCg (1mg) and CH_3SNa (1.5mg) were incubated in a sealed vial at pH 7.5 (37°C). A sample was withdrawn from the mixture and 0.12 N HCL was added before the HPLC analysis

Purification of the reaction mixture by preparative HPLC afforded three compounds not previously described in the literature. Their structures were established by NMR spectroscopy and SIMS (Yasuda and Arakawa, 1995). Compound 1 (HPLC retention time 13.3 min) was identified as 2'-methylthio EGCg. Compounds 2 and 3 (co-eluted, HPLC retention time 17.8 min) were identified as 2',6'-dimethylthio EGCg and 2'-

methylsulphinyl-6'-methylthio EGCg respectively. The third HPLC elution peak (retention time 15.6 min) was assumed to be an epimer at the position 3 of compound 2.

Richard *et al.* (1991) have reported that the B ring of EC was oxidised with apple polyphenol oxidase to give an ortho-quinone, and that cysteinyl addition compounds were obtained in the presence of cysteine. Haslam *et al.* (1992) have illustrated quinone-tanning between ortho-quinone intermediates and nucleophilic -NH_2 and -SH groups in a protein. Natural deodorants with an ortho-quinone structure have been isolated in small amounts from thyme (Nakatani *et al.*, 1989; Miura *et al.*, 1989). These data suggest that deodorization follows an oxidative mechanism. This was further stressed by the suppression of the deodorizing reaction in our assay at pH values lower than 5, and by the partial restoration upon addition of a polyphenol oxidase. Addition of polyphenol oxidase also increased the deodorizing activity at pH 7.5.

The effect of atmospheric oxygen in our deodorizing assay system was examined. Under the ordinary conditions of our CH_3SH deodorizing assay, the amount of EGCg decreased with increasing time, and compound 1 appeared in the mixture (Fig.2) (Yasuda and Arakawa, 1995).

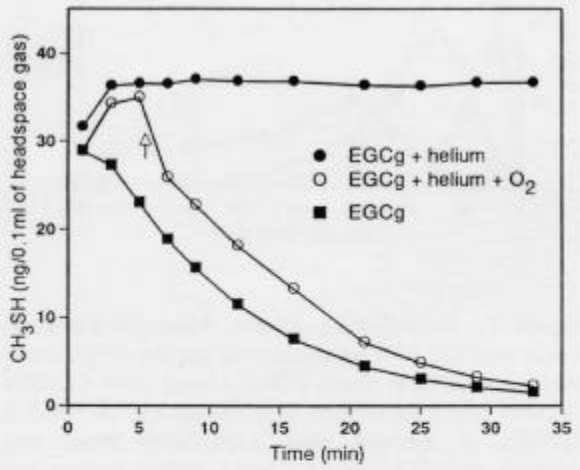

Figure 2. Effect of oxygen and helium gas on the CH_3SH deodorizing action of EGCg. Both EGCg (1mg) and a 25 ppm CH_3SNa solution (0.5ML) were incubated in a sealed vial at pH 7.5 (37°C). An arrow shows when oxygen was introduced into the sealed vial.

However, under anaerobic conditions with helium gas, EGCg was not degraded and no deodorizing activity was observed. The injection of O_2 into the sealed tube restored the deodorizing activity of EGCg. These observations led us to propose a schema (see schema) of reaction between EGCg and CH_3SH.

Tri-phenolic hydroxyl groups in B ring of EGCg is oxidised with atmospheric oxygen to an ortho-quinone intermediate. CH_3SH then reacts with the quinone by a 1,4 or 1,6-addition to afford the 2'-methylthio EGCg derivative. CH_3SH similarly reacts with compound 1 to afford compound 2. When compound 1 was reacted with CH_3SH a similar pattern to that shown in Fig. 1 was observed. Compound 3 derives from the oxidation of compound 2 and could also derive from compound 5.

Deodorizing activity of compounds 1, 3 and 5 (produced by oxidation of 1 with hydrogen peroxyde were compared to those of EGCg and to sodium copper chlorophyllin, a commercially available oral deodorizer (Yasuda and Arakawa, 1995). At identical concentrations, the activities of EGCg and sodium copper chlorophyllin were equivalent, whereas the activities of all metabolites were higher. In particular, the deodorizing activity of compounds 1 and 5 were twice and three times as strong as that of EGCg, respectively. We assume that the introduction of a methylsulphinyl group at the 2' position resulted in a lower electron density in the B ring and activation of the 6' position on the quinone towards nucleophilic attack. It is interesting to note that compounds 2 and 3, already substituted at the 2' and 6' positions, had only moderate activity possibly due to the reaction of CH_3SH with the galloyl group.

Dimethyl disulphide (CH_3SSCH_3) was not found in any deodorizing assay. The deodorizing effect of EGCg against CH_3SH may thus be principally due to the addition reaction described above.

Acknowledgement

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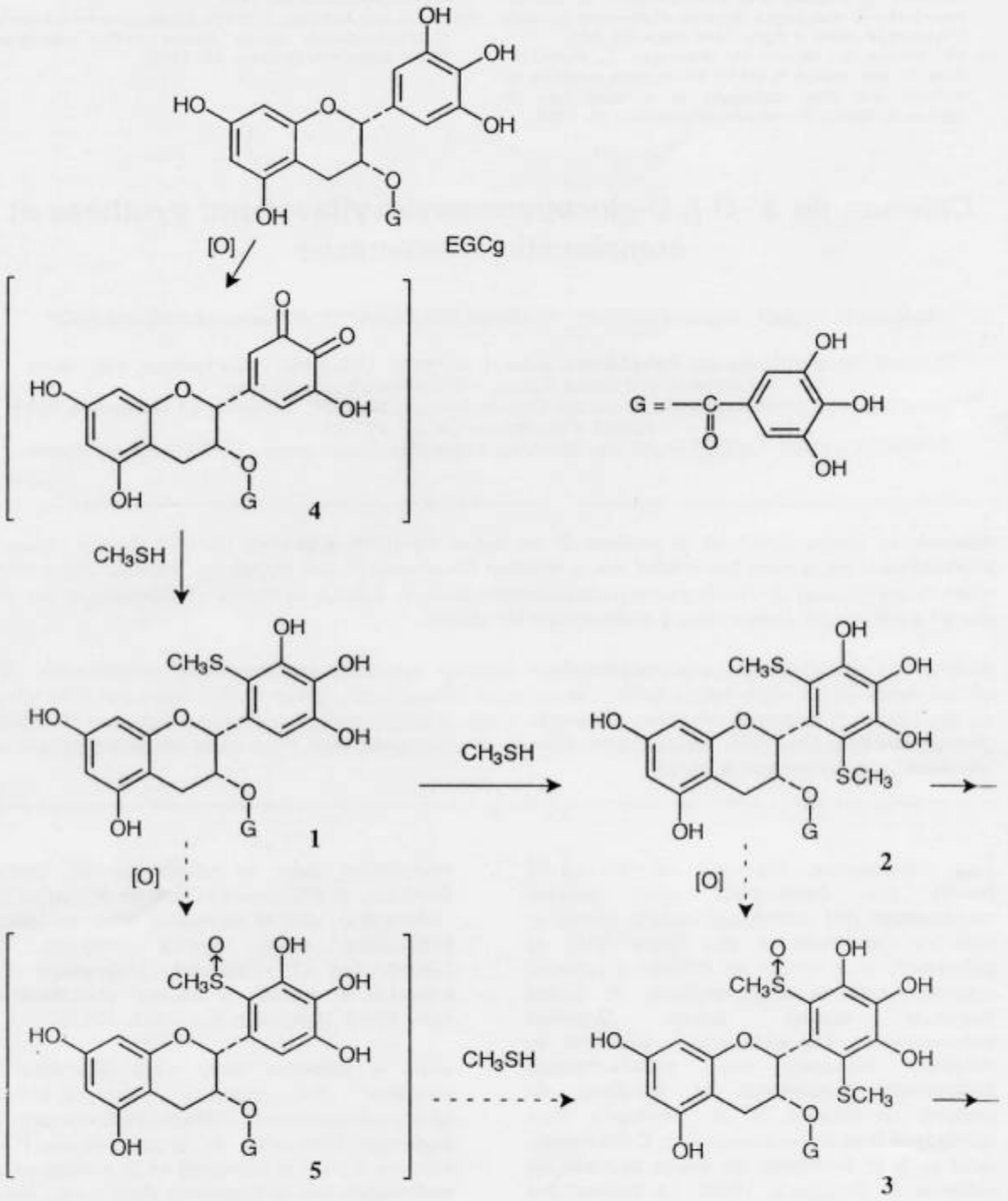

Schema

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Chlorure de 3'-*O*-β-D-glucopyranosyloxyflavylium: synthèse et complexation moléculaire

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Résumé: La glucosylation de la position 3' du cation flavylium augmente l'acidité de ses groupes phénoliques mais produit peu d'effet sur la réaction d'hydratation. Les complexes moléculaires formés entre le chlorure de 3'-*O*-β-D-glucopyranosyloxyflavylium, la caféine et l'acide chlorogénique ont été mis en évidence par spectroscopie d'absorption UV-visible.

Abstract: *3'-O-β-D-glucopyranosyloxyflavylium chloride: synthesis and molecular complexation.* 3'-Glucosylation of flavylium cation leads to an increase in its phenolic group acidity, but it has little effect on the flavylium hydration reaction. Molecular copigmentation complexes formed between 3'-O-β-D-glucopyranosyloxyflavylium chloride and caffeine or chlorogenic acid have been identified by use of UV-visible absorption spectroscopy.

Les anthocyanes, pigments naturels de la famille des flavonoïdes, sont souvent responsables des colorations rouges, bleues et violettes des fruits et des fleurs. Elles se présentent sous forme de dérivés glycosylés polyhydroxylés et polyméthoxylés du cation flavylium encore appelé 2-phényl benzopyrylium. Les anthocyanes subissent en solution aqueuse des transformations structurales (hydratation et transferts de proton), conduisant à la formation d'un hémiacétal **B** et de chalcones **E** et **Z** (incolores), ainsi qu'à la formation de bases quinoniques colorées **A** (Brouillard, 1982). La couleur des anthocyanes est stabilisée par le phénomène de copigmentation qui est une interaction moléculaire entre une anthocyane et une molécule organique incolore ayant une partie plane riche en électrons π, et que l'on appelle copigment. La copigmentation consiste en la formation d'un complexe d'empilement vertical entre le copigment et l'anthocyane. En milieu faiblement acide, la copigmentation est en compétition avec la solvatation du cation flavylium, et déplace l'équilibre de formation de l'hémiacétal vers la formation d'un complexe moléculaire entre formes colorées de l'anthocyane et copigment, phénomène qui intensifie et modifie la couleur (Brouillard *et coll.*, 1989; Dangles et Brouillard, 1992).

Dans la présente note, nous décrivons la synthèse du chlorure de 3'-*O*-β-D-glucopyranosyloxy-4',7-dihydroxyflavylium **1**, ainsi que l'influence du groupe glucosyl en position 3', sur la réactivité et la complexation moléculaire des anthocyanes dans l'eau. Nous avons utilisé deux copigments la caféine et l'acide chlorogénique. L'étude physico-chimique a été faite par spectroscopie UV-visible.

La 3,4-dihydroxyacétophénone **3** est obtenue par réduction de **2** en présence de poudre de zinc dans un mélange tétrahydrofuranne-acide acétique 4:1 (Hendrickson et Kandall, 1970). Ensuite, le groupe hydroxyle en position 4 est