α- and β-Guttiferins

The distribution of kindred antistaphylococcal principles in every tissue, particularly in the seed coat and gamboge, the resinous exudation, of *Garcinia morella* Desr. was inferred from chromatographic, optical and spectral evidence[1–4]. Next to morellin[5], 'guttiferin' soluble in aq. sodium carbonate forms the major component fraction[6] of extracts of the seed coat from which *α-guttiferin*(I), $C_{33}H_{38}O_8$, m.p. 113–115° [$(\alpha)_D^{26} = -475°$ (c, 1.5 in $CHCl_3$); λ_{max}^{EtOH} 278 mμ ($\log K = 1.57$) and 360 mμ ($\log K = 1.327$)] has been separated from *γ,δ* and possibly other guttiferins[7], as a sparingly soluble orange yellow crystalline *pyridine-complex* $C_{33}H_{38}O_8 \cdot C_5H_5N$, m.p. 115–117° [$(\alpha)_D^{26} = -561.2°$ (c, 1.496 in $CHCl_3$); λ_{max}^{EtOH} 278 mμ ($\log K = 1.5763$) and 360 mμ ($\log K = 1.3385$)].

Likewise, the allied pigment *β-guttiferin* (II) $C_{29}H_{36}O_6$ or $C_{33-34}H_{38-40}O_7$, m.p. 86–91° [$(\alpha)_D^{24} = -698.2°$ (c, 1.063 in $CHCl_3$); λ_{max}^{EtOH} 291 mμ ($\log K = 1.62$) and 362 mμ ($\log K = 1.53$); (II) may be identical with α-gambogic acid[8]] is the main constituent crystallizing as a *complex* with one molecule of pyridine melting at 148–149° [$(\alpha)_D^{24} = -624.3°$ (c, 1.276 in $CHCl_3$); λ_{max}^{EtOH} 290 mμ ($\log K = 1.7803$) and 362 mμ ($\log K = 1.7076$)] from ether soluble Indian, Malayan and Indonesian gamboge. A prerequisite for formation of these complexes is the presence of the acidic phenol and enol groups (*vide infra*). The

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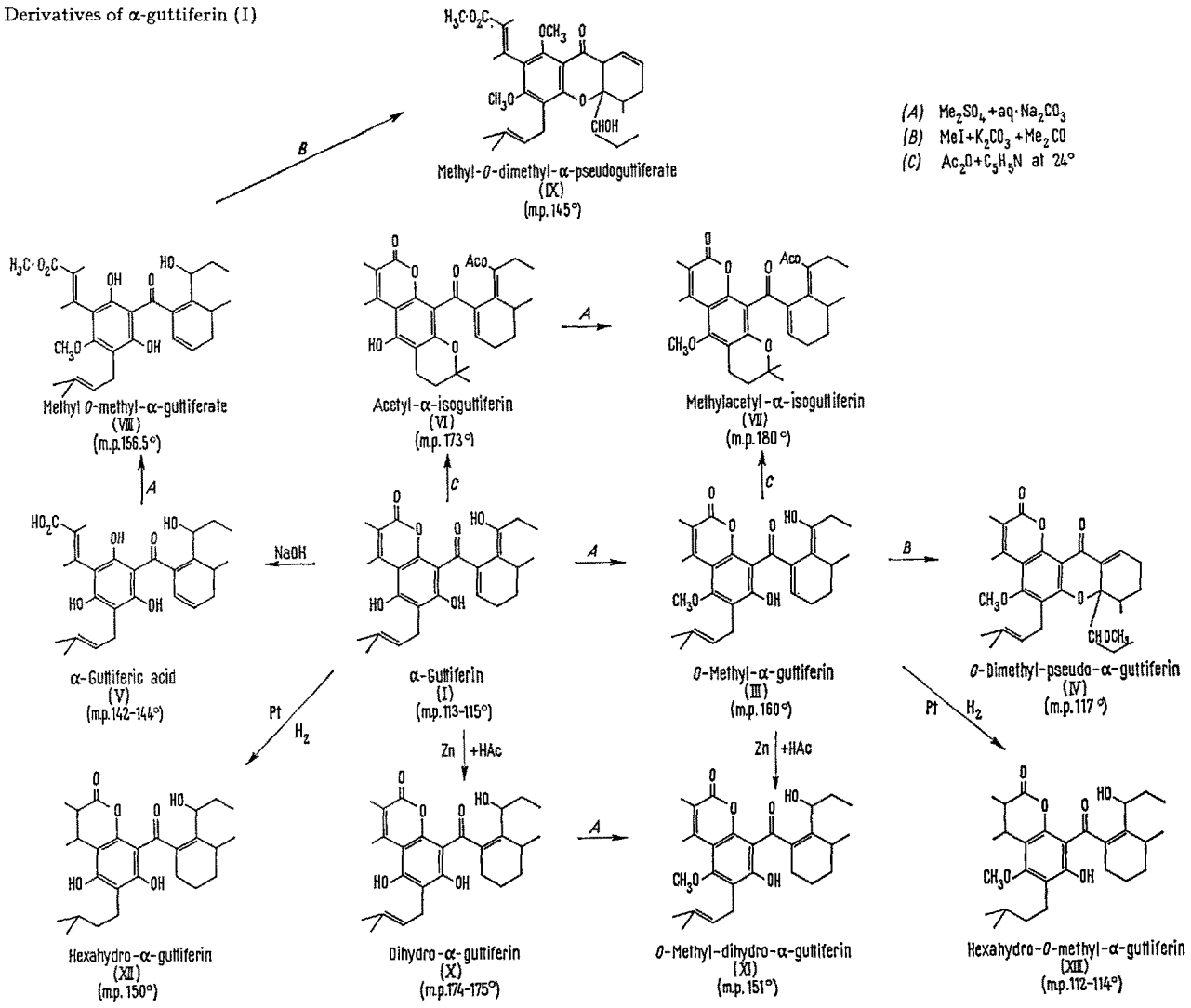
[6] D. Rajagopal Rao, K. V. Nageswara Rao, and P. L. Narasimha Rao, *Symposium on Antibiotics* (Published by the Council of Scientific and Industrial Research, India 1956), p. 46.
[7] V. S. Gupta and P. L. Narasimha Rao, unpublished results.
[8] M. Amorosa, Ann. chim. *45*, 40 (1955). – M. Amorosa and L. Liparini, Ann. chim. *45*, 977 (1955). – The nomenclature of several constituent fractions of gamboge (none of them obtained in a pure condition till recently) described by various workers since Newmann (Chym. Med. 1751) has been far from satisfactory and it thus seems preferable to designate this compound as β-guttiferin in line with the other constituents of the tree even though it may prove identical with α-gambogic acid.

Derivatives of α-guttiferin (I)



kinship of (I) and (II) to morellin[9] $C_{33}H_{38}O_7$ and moreollin[10] $C_{33}H_{40}O_8$, two known pigments from the seed coat is apparent from the chemical and biological properties[2,3,6].

The characteristic formation of analogous derivatives from (I) and (II) is, however, marked by the facile crystallisation of those of (I) presented in the Figure. (I) and (II) contain no methoxyl or free carboxylic groups. Three of the oxygen atoms in (I) are demonstrable as acidic phenol (solubility in aq. sodium carbonate), enol (formation of red copper complex analogous to isomorellin[10]) and a tertiary hydroxyl group. Two more, phenolic and carboxyl carbonyl, contribute to an α,β-unsaturated δ-lactone system. A hindered phenol, a carbonyl group para to the acidic phenolic moiety and a possible oxide ring make up for the remaining three. The presence of three double bonds is indicated by catalytic reduction of (I) and (III) to the corresponding *hexahydro-compounds* (XII) and (XIII). (I) reacts with carbonyl reagents, but the products do not seem typical. With semicarbazide and hydroxylamine, (I) forms $C_{34}H_{41}O_8N_3$, m.p. 255° (sinters at 180°), and $C_{33}H_{40}O_8N_2$, m.p. 172–174°, respectively. Similarly the *methyl ether* (III) reacts with semicarbazide and phenylhydrazine to give $C_{35}H_{43}O_8N_3$, m.p. 128–132°, and $C_{40}H_{46}O_7N_2$, m.p. 153–155°, respectively. The three phenolic groups referred to are, apparently, part of phloroglucinol recognized among the products of alkali fusion of (I) and (II) containing methylheptenol, homophthalic and isovaleric acids which are also formed by morellin[11,12]. (I) does not react with diazoaminobenzene nor couple with diazotized sulphanilic acid, thus indicating the presence of a fully substituted phloroglucinol structure as represented in the Figure. By the action of alkalies, the lactone rings in (I) and (II) are opened and the liberated acids changed to the *trans* form, *guttiferic acids* (cf. V), accompanied by a shift in the double bonds affecting the enolic group. Physico-chemical data which include ultra-violet and infrared spectra and colour reactions support the isomeric changes (vide Fig.) occurring during methylation of (I) and (III) to the *dimethyl ether* (*IV*) and of (VIII) to (IX) by method (B) *viz.*, with methyl iodide in acetone in presence of potassium carbonate, as well as during acetylation to the *monoacetyl-compound* (VI) with pyridine and acetic anhydride (method C).

Apart from formation of analogous derivatives with similar spectral characteristics, the close relationship between (I) and (II) is further suggested by oxidation with sodium hypobromite to the same *bromo-acid* m.p. 199 to 200° [λ_{max}^{EtOH} 230 mμ ($\log K = 1.625$) and 312 mμ ($\log K = 1.56$] still under investigation. These and other degradative reactions of (I) and (II) will be discussed elsewhere.

Like morellin[9,12,13], (I) and (II) are specifically active against Gram-positive bacteria and the bacteriostatic effect against *Micrococcus pyogenes* var. *aureus* (MIC 0.1–1 μg/ml) is reversed by methionine, albeit to a lesser extent. Further, they show similar cross reactions. The presence of blood serum on their antibacterial activity is less pronounced and experimental staphylococcal infections in mice are controlled by (I) and (II).

Zusammenfassung. Die Isolierung, Charakterisierung und antibiotischen Eigenschaften von α-Guttiferin (I), $C_{33}H_{38}O_8$ (Smp. 113–115°), aus den Samenhülsen und vom nahe verwandten β-Guttiferin (II), $C_{29}H_{36}O_6$ oder $C_{33-34}H_{38-40}O_8$ (Smp. 86–91°) (möglicherweise identisch mit α-Gambogasäure), aus Gummigutt, dem harzigen Sekret von *Garcinia morella*, wurden beschrieben und ihre Verwandtschaft zu Morellin und Moreollin, den beiden bekannten Pigmenten der Samenhülsen, dargelegt. Für I wurde eine Partialstruktur vorgeschlagen. Die Bildung derselben komplexen bromhaltigen Säure vom Smp. 199 bis 200° unter der Einwirkung von Natriumhypobromit auf I und II spricht ferner für deren nahe strukturelle Verwandtschaft.

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[10] The revised molecular formula $C_{33}H_{40}O_8$ (cf. [4]) conforms to its formation from morellin by the action of alcoholic potassium acetate and its change to isomorellin by pyridine (P. L. NARASIMHA RAO et al., unpublished results).

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Estrogenic Activity and Steric Hindrance to Coplanarity of Alkyl Substituted 4,4′-Dimethoxystilbenes[1]

Since the pioneering work on artificial estrogens by COOK and DODDS[2], a great number of compounds have been tested for estrogenic properties[3]. Comparison of activity is difficult because of the various assay procedures that have been used. Compounds showing activity comparable to the natural female sex hormones are to be found in the classes of stilbene derivatives (stilbestrol), the diphenylethane derivatives (dienestrol and hexestrol) and among the doisynolic acids. The most active substances have in common that the molecules consist of a rather large, rigid, skeleton with, in most cases, a hydroxyl group at both ends. Possibly, there exists an optimum distance for the two hydroxyl groups[4]. From the large differences in biological activity among the α,α′-dialkyl-4,4′-dihydroxystilbenes, it is clear that also other structural features are important. OKI studied stilbene derivatives with halogeno and thiomethyl groups and concluded, from spectroscopical data, that the thickness of the molecule may be a critical factor[5]. We have measured the estrogenic properties of a series of alkyl substituted 4,4′-dimethoxystilbenes[6], in which other than steric effects are minimized.

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