in different solvents, do not show $\Delta\varepsilon/\varepsilon$ values consistently higher than those shown at pH 9, these reaction conditions are the most suitable for CD measurements. When free L-cysteine was made to react with 2-fluoro-3-nitropyridine, the positive band at 367 nm and the negative band at 425 nm increase simultaneously as a consequence of S→N aryl migration (Figure 6). As a result of these studies it may be postulated that, when thiol and amino moieties are in a suitable relative position, they react as a common functional group with electrophilic compounds. This implies that a selective modification of –SH groups is not possible in these cases. However, this does not complicate the configurational assignments, since both CD bands appear to be diagnostic of the optical configuration of cysteine residues.

On the other hand, if methylisothiocyanate is allowed to react at pH 4–6 with cysteinyl peptides, selective S-methylthiocarbamoylation takes place[22]. The derivatives exhibit a weak UV-absorption band at about 325 nm in water, along with bands of greater complexity at about 270 and 250 nm (Figure 7). When the dithiocarbamate group is in a dissymmetrical environment, these transitions present optical activity; Figure 8 illustrates the CD curve of S-methylthiocarbamoyl-glutathione at pH 4. The sign of the 320 nm CD band is strictly solvent-dependent, as shown in other thiocarbonyl compounds[24]; therefore it is not automatically transferable to organic or aqueous-organic solutions. Hence, since the 320 nm CD band presents a high dissymmetry factor, its sign at pH 4–6 can be used to determine the optical configuration of the α-carbon atom of cysteine in peptides, being positive for L-cysteinyl derivatives and negative for D-derivatives. In the experimental conditions employed, no S→N shift of –C(=S)-NHMe group has been observed.

Therefore, on the basis of chirospectroscopic properties of S-3-NPyr- and S-methylthiocarbamoyl-cysteinyl derivatives, it is possible to conclude that both chromophoric derivatives are extremely valuable, so far as the configurational assignment of cysteine in naturally occurring peptides is concerned.

Zusammenfassung. Es wird eine neue Methode der stufenweisen Konstitutionsaufklärung von Polypeptiden vorgeschlagen, mit einer gleichzeitigen Bestimmung der Aminosäuren-Konfiguration. Es ergab sich, dass man im Falle der freien Aminosäuren die Chromophorengruppe des Carboxyls, gegebenenfalls auch jene der Seitenketten benützen kann. Für die Peptide sind neue Chomophorenderivate N-endständiger Aminogruppen und der SH-Funktion des Cysteins vorgeschlagen worden.

SPECIALIA

Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. – El responsable de los informes reducidos, está el autor.

On the Biosynthesis of Neoflavanoids

In continuing our studies on the biosynthesis of 4-phenyl coumarins[1] (neoflavanoids[2]) we investigated the biogenetic relationship between calophyllic acid (Ia)[3] and inophyllolide[3] (II), two minor constituents of *Calophyllum inophyllum* (Guttiferae) seeds. We wished to establish whether it is the acid (Ia) or the lactone (II) which is biosynthetized first. The comparison of specific activities after incorporation of a common radioactive precursor should resolve this question, since the intermediate should be the more radioactive compound[4].

We therefore administered 3-^{14}C phenylalanine, an established precursor of the C_9 unit of 4-phenyl coumarins[4], to young shoots of *C. inophyllum* in 2 separate experiments (lasting 1 and 2 weeks, respectively). Calophyllic acid (Ia) and inophyllolide (II) were isolated by repeated chromatography and crystallized to constant radioactivity[5]. The results (dpm/mM) of both experiments are given in the Table. They reveal an approximately 8-fold specific activity for (Ia) [counted as its more soluble methyl ester (Ib)] than for (II). This suggests that calophyllic acid (Ia) is the biogenetic precursor of inophyllolide (II)[6].

In order to control the above results we fed, in a short-term experiment (24 h), U-^{14}C isoleucine, a very efficient precursor of the 2,3-dimethyl chromanone ring[7]. Again,

[1] G. Kunesch and J. Polonsky, Phytochemistry *8*, 1221 (1969).
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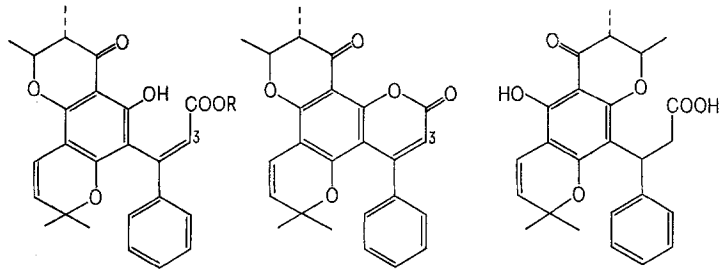


Ia: R=H
Ib: R=CH_3

II III

compound (Ib) was found to be 5 times more active than the lactone (II).

The compound (Ia) possesses a hydroxyl group in the δ-position of the carboxyl group. Similar stable δ-hydroxy acids have been isolated from several other *Calophyllum* species[8,9]. The higher specific activity might be explained by the stereochemistry (E) of the α, β double bond of the acid. In this case, an isomerization step would precede the lactonization step and reasonably explain the higher label of the acid. A similar biological double bond isomerization has been observed in coumarin biosynthesis[10].

In order to establish unambiguously the stereochemistry of the α, β double bond of calophyllic acid (Ia), we have studied comparatively the Nuclear Overhauser Effect (NOE) of methyl calophyllate (Ib) and inophyllolide (II). Because of the rigid nature of the latter compound, a strong interaction between the proton at C-3 and the neighbour aromatic protons was expected. In fact, irradiation of the aromatic protons at approximately 7.2 ppm causes an increase of the intensity of the proton at C-3 of 22%. For compound (Ib), a NOE of 21% was observed under the same experimental conditions for the corresponding proton, thus establishing unambiguously for the α, β double bond of calophyllic acid (Ia) the stereochemistry (Z).

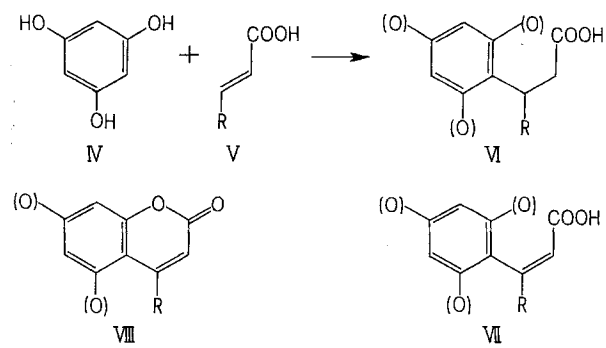
The absence of spontaneous lactonization of calophyllic acid may be explained by the strong hydrogen bond of the phenolic OH with the carbonyl group of the *trans* 2,3-dimethyl chromanone ring (for example, the OH proton absorbs at 12.5 ppm and does not react with diazomethane).

So, as previously stated, the above results suggest a biogenetic sequence involving calophyllic acid (Ia) as the immediate precursor of inophyllolide (II).

On the basis of this assumption, the following general scheme of biosynthesis based on an earlier proposal by

	1st incorporation (after 1 week)	2nd incorporation (after 2 weeks)
Ia	37.93×10^4	50.2×10^4
II	5.15×10^4	6.27×10^4
Ia/II	7.37	8.00

Scheme A



IV V VI VIII VII

R = C_6H_5 or n-alkyl

[8] G. H. STOUT, G. K. HICKERNELL and K. D. SEARS, J. org. Chem. *33*, 4191 (1968).
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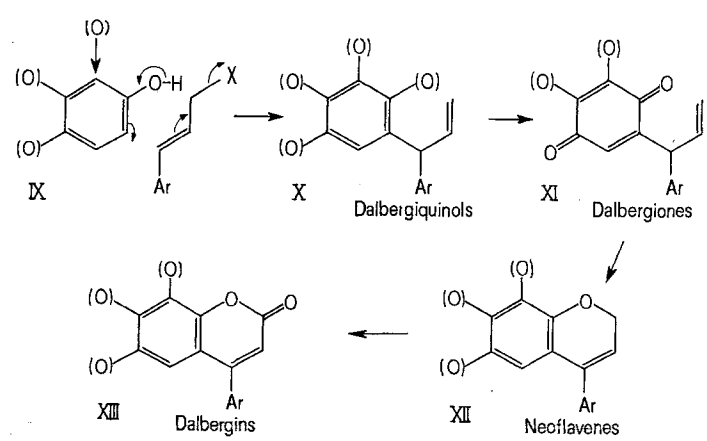
[12] No exception to the 1, 3, 5-oxygenation pattern of the polyketide unit has so far been recognized among Guttiferae constituents.

Scheme B



SESHADRI[11] may be envisaged for 4-substituted coumarins and related constituents of Guttiferae (scheme A): β-addition of phloroglucinol[12] (IV) to cinnamic acid (V) (or its biological equivalent) leads to acids of type (VI); a representative of this type of compound, chapelieric acid, has been isolated recently for the first time from a *Calophyllum* species[13]. The possibility of this reaction in vitro has been demonstrated[14]. Dehydrogenation leads to compounds like (VII) which on lactonization gives (VIII). Replacement of cinnamic acid by even-numbered α, β-unsaturated fatty acids (or their biological equivalents) would account for the biogenesis of compounds like apetalic acid[15] as well as for the biosynthesis of the 4-alkyl-coumarins, which co-occur frequently in *Calophyllum* species[16,17].

According to OLLIS and GOTTLIEB[18] the 4-phenyl coumarins could be formed by alkylation of a phenolic unit by cinnamyl pyrophosphate and the reactions shown in scheme B. This proposal is based on considerable phytochemical evidence and has recently been substantiated by convincing in vitro experiments[19]. However, this theory is not consistent with the existence of intermediates like calophyllic acid (Ia) in the biogenesis of inophyllolide (II).

Although results obtained from in vivo experiments in higher plants must be interpreted with caution, different pathways of neoflavanoid biosynthesis in quite different botanical families, such as the Guttiferae and Leguminosae, might best account for the present knowledge in this field. The following major differences in structural features support this suggestion: 4-phenyl coumarins isolated from the Guttiferae generally possess an acyl and an isoprenoid substituent on the polyketide unit, which are absent in the Leguminosae family, whereas the wide variety of compounds of types (X), (XI) and (XII) has not so far been isolated from Guttiferae.

It may be noted that in a recent paper OLLIS[20] does not exclude the possibility of different biogenetic pathways for the neoflavanoids in the Guttiferae and Leguminosae families.

Résumé. Les hypotheses sur le mécanisme de la biosynthèse des néoflavanoïdes sont discutées sur la base de résultats de nos expériences d'incorporation dans le *Calophyllum inophyllum*.

J. GAUTIER, A. CAVE, G. KUNESCH and J. POLONSKY[21]

Institut de Chimie des Substances Naturelles, C.N.R.S., F-91 Gif-sur-Yvette (France), 6 January 1972.

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The Role of Phenolic Acids in the Browning, Spontaneous Heating and Deterioration of Stored Soybeans

The spontaneous browning and heating of stored soybeans causes considerable damage and is, in consequence, an economical and chemical problem of significance. We therefore undertook the present investigation to elucidate its mechanism and devise a way of its early detection. A theory had been advanced previously by MILNER and THOMPSON[1] that the main process involved was a Maillard-type sugar-protein interaction[2,3], the starting heat being possibly provided by fungal growth. This would, however, not explain why spontaneous heating and browning occurs so much more easily in soybeans than in any cereal stored under the same conditions.

In the course of experiments connected with a previous communication[4], we found that when a dried aqueous extract of browned soy-bean flakes was applied to a polyamide column, a brown pigment came off together with the water wash. This pigment, which made up almost all the coloured material present in the extract, gave a typical reaction for phenols with ferric chloride solution. Therefore, a reaction involving phenols should play a role in the browning of soybeans.

In order to investigate this phenomenon more thoroughly, a systematic analysis was carried out. Soybeans, finely ground in a blender, were extracted with ether in a Soxhlet extractor and the ether solution extracted for acidic, weakly acidic and basic substances[5].

The acidic, bicarbonate-soluble fraction obtained from 'browned', but not from sound soybeans, contained a number of phenolic substances, as evidenced by paper chromatography (Figure), thin-layer chromatography and the Folin-Ciocalteu reaction[6]. Diazotised sulfanilic acid (Pauly reagent) was used for visualization[7].

In order to get a quantitative approach we devised the following procedure, based on that of SINGLETON and ROSSI[8]: 50 g of soybeans were ground and extracted in a Soxhlet extractor with diethyl ether. The ether was brought to a volume of 50 ml and extracted with 20 ml bicarbonate solution (5%). One ml of this was diluted with 1 ml of the distilled water and 1 ml of the Folin phenol reagent (BDH-diluted 1:4) was added, followed after 3 min

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