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*.

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[13] E. GUERREIRO, G. KUNESCH and J. POLONSKY, Phytochemistry *10*, 2139 (1971).
[14] J. D. SIMPSON and H. STEPHEN, J. chem. Soc. *1956*, 1382.
[15] T. R. GOVINDACHARI, D. PRAKASH and N. VISWANATHAN, Tetrahedron Lett. *1967*, 4177.
[16] L. CROMBIE, D. E. GAMES and A. MCCORMICK, J. chem. Soc. (C), *1967*, 2545.
[17] S. K. NIGAM, C. R. MITRA, G. KUNESCH, B.C. DAS and J. POLONSKY, Tetrahedron Lett. *1967*, 2633.
[18] W. D. OLLIS and O. R. GOTTLIEB, Chem. Commun. *1968*, 1396.
[19] L. JURD, Tetrahedron *25*, 1407 (1969).
[20] W. D. OLLIS, An. Acad. brasil. Ciênc. *42*, 9 (1970).
[21] We thank the C.E.A. (Saclay) for a grant towards the purchase of labelled compounds, Mr. G. HENRY for technical collaboration and the 'Comité de l'Accueil du Phytotron' for culture facilities.

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].

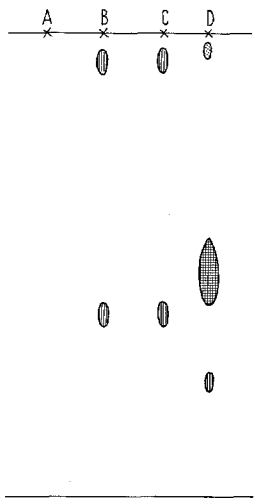
The acidic, bicarbonate-soluble fraction obtained from 'browned', but not from sound soybeans, contained a number of phenolic substances, as evidenced by paper chromatography (Figure), thin-layer chromatography and the Folin-Ciocalteu reaction[6]. Diazotised sulfanilic acid (Pauly reagent) was used for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

[1] M. MILNER and J. B. TOMPSON, J. agric. Food Chem. *2*, 303 (1954).
[2] L. C. MAILLARD, C. r. Séanc. Acad. Sci, Paris *154*, 66 (1912).
[3] G. R. ELLIS, Adv. Carbohyd. Chem. *14*, 63 (1959).
[4] A. FRIEDLANDER and B. SKLARZ, Experientia *27*, 762 (1971).
[5] J. PASTO and C. H. JOHNSON, *Organic Structure Determination* (Prentice Hall, Englewood Cliffs, N.J. 1969).
[6] O. FOLIN and C. CIOCALTEU, J. biol. Chem. *73*, 627 (1927).
[7] R. GRIMMETT and E. L. RICHARDS, J. Chromat. *20*, 171 (1965).
[8] V. SINGLETON and J. A. ROSSI JR., Am. J. Enol. Vitic. *16*, 144 (1965).

by 1 ml of sodium carbonate solution 10%. Spectro-photometric readings were effected 90 min later at wavelength 770 nm. When the optical density exceeded 1,800 the reaction was repeated on a 10-fold dilution of the original bicarbonate extract; the optical density, so obtained was multiplied by 10. Optical densities obtained this way were used to correlate phenolic acid content with other factors, as described herewith, and are temporarily defined as phenolic acid index.

It can be seen, from the figure, that no Pauly reactive substances are obtained when the phenolic acid index is 0.827 or less. (This reading is probably due to uric acid, a natural component of soybeans[9], which also gives a positive Folin reaction, but does not couple with Pauly reagent). Table I gives indices for fresh and damaged soybeans. The fact that the paper chromatogram of the most deteriorated sample D shows a different pattern from samples B and C indicates that the browning process, in terms of phenolic acid formation, is a complex one. The relatively high reading for mouldy, but not discoloured beans in the Table I seems to indicate that moulds are the cause of an increase in phenolic acids. It is clear from the above that the phenolic acid index is an indicator for starting deterioration long before any visible discoloration appears on the bean.



Paper chromatography of phenolic acids, extracted from soybeans in various stages of deterioration. (Solvent 20% aqueous KCl, visualization Pauly reagent[7]. A) sound beans, phenolic acid index 0.827; B) slightly browned beans, phenolic acid index 6.60; C brown beans; phenolic acid index 6.75; D) brown-black beans, phenolic acid index 17.00.

Table I. Increase in phenolic acids with deterioration

	Phenolic acid index
Sound beans	0.520
White, visibly mouldy beans	1.770
Black beans sample 1	12.20
Black beans sample 2	8.20

Table II. Increase of phenolic acid index with temperature

Date of sampling		22.4	2.5	1.6
Test Point 1	Phenolic acid index	0.740	1.34	3.15
	t, °C	21	22.5	24
Test Point 2	Phenolic acid index	0.950	1.51	2.08
	t, °C	23	25	27
Test Point 3	Phenolic acid index	0.450	1.44	5.70
	t, °C	—	30.5	37

Table III. Temperature, phenolic acid index and percent germination in stored soybeans

T, °C	Phenolic acid index	Germination %
18	0.450	80
18	0.950	89
25	1.04	62
25	1.66	76
25	1.4	83
25	1.34	0
25	1.90	0
25	2.08	0
25	3.15	0
25	4.35	0

Normal soybeans: 80–90% germination

Testing a storage bin at various points at various times, we found increases in phenolic acid index with a rise in temperature. These findings are summarized in Table II.

An interesting correlation was found between the phenolic acid index and germination. At 25 °C germination power decreased considerably when the phenolic acid index reached 1.04, and went down to zero, when it reached 1.90. The actual measurements are shown in Table III.

From the data in our possession, it is not yet possible to establish the exact role of phenolic acids in the inhibition of germination. MAYER[10] as well as BERRIE et al.[11] have described a number of fungus-produced phenolic lactones that inhibit the germination of lettuce seeds, while MACKO et al.[12,13] showed 2 derivatives of cinnamic acid to be inhibitors of the germination of Uredospores.

There are a number of ways in which phenolic acids could participate in browning and heating: They could, like any phenol, form brown humus-like materials by condensing with sugars, in analogy to Baekeland's phenol-formaldehyde reaction[14]; cinnamic acids could be polymerized by a free radical mechanism, possibly catalyzed by traces of metals. Both reactions are exothermic and could contribute to 'spontaneous' heating. The possible formation of melanine through the action of a fungal polyphenol oxidase[15] can also not be overlooked.

It seems that fungi or other plant pathogens are the cause of the formation of phenolic acids in soybeans. This

[9] We are grateful to Dr. S. NEUMARK for this information.
[10] A. M. MAYER, Israel J. Bot. *13*, 41 (1964).
[11] A. M. M. BERRIE, W. PARKER, B. A. KNIGHTS and M. R. HENDRIE, Phytochemistry *7*, 567 (1968).
[12] V. MACKO, R. C. STAPLES, H. GERSHON and J. A. A. RENWICK, Science *170*, 539 (1970).
[13] V. MACKO, R. C. STAPLES, P. J. ALLEN and J. A. A. RENWICK, Science *173*, 835 (1971).
[14] C. S. MARVEL and E. C. HORNING in H. GILMAN, *Organic Chemistry*, 2nd edn. (Wiley, New York 1943), vol. 1.
[15] J. S. FRUTON and S. SIMMONDS, *General Biochemistry*, 2nd edn. (Wiley, New York 1958), p. 830.

can be inferred from our findings, in addition to the large amount of evidence accumulated in the literature. Some of the phytoalexins mentioned by CRUICKSHANK[16] are derived from phenolic acids. KLARMAN[17] isolated phenolic substances from soybeans infected with phytophtora megasperma; so did BIEHN, WILLIAMS and KUĆ[18] after infecting the beans with various fungi.

This paper does not deal with the physiological and toxicological sides of the phenomenon. However, in view of the ultimate aim of human consumption, these aspects have to be kept in mind, as clearly pointed out by SINGLETON and KRATZER[19].

Zusammenfassung. Beim spontanen Bräunen und Erhitzen von gelagerten Soyabohnen bilden sich Phenolsäuren, welche eine aktive Rolle in Entstehen des Lagerschadens spielen und auch als dessen Frühindikator dienen können.

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Stored Products Research Laboratory, Ministry of Agriculture, P.O. Box 15030, Jaffa (Israel), 22 November 1971.

[16] J. A. M. CRUICKSHANK, A. Rev. Phytopath. *1*, 351 (1963).
[17] W. L. KLARMAN and J. B. SANDFORD, Life Sci. *1*, 1095 (1968).
[18] W. L. BIEHN, J. Kuć and E. B. WILLIAMS, Phytopathology *58*, 1255 (1968).
[19] V. L. SINGLETON and F. H. KRATZER, J. agric. Food Chem. *17*, 497 (1969).
[20] The authors wish to express their appreciation to Prof. A. BONDI for helpful suggestions. They also wish to thank Misses FRIEDA RABINOWITZ and MIRI KEKUN for skilful technical assistance.

Synthesis and Stereospecific Synthesis of some Alkyl-5-Amino-1-Glycofuranosyl Imidazole-4-Carboxylates Related to Intermediates in Purine Nucleotide de novo Biosynthesis

The aminoimidazolecarboxylic acid ribotide C-AIR (I) is an important intermediate in the biosynthesis de novo of purine nucleotides[1], and is of value for the synthesis of acyclic intermediates in the same pathway. We have synthesized the nucleotide previously[2] by phosphorylation of the isopropylidene ribonucleoside (IIa), subsequent removal of the isopropylidene group with acid, and hydrolysis of the ester with alkali. The imidazole ester (IIa) was obtained by the reaction of 2, 3, 5-*tri*-*O*-benzoylribofuranosylamine (III) with the formimidate (IVa), hydrolysis of the derived tribenzoyl nucleoside, and condensation of the resulting nucleoside with dimethoxypropane and tosic acid, or by condensation of the 2, 3,-*O*-isopropylidene ribofuranosylamine tosate (V)[3] with the same reagent (IVa) in the presence of base.

Subsequent reactions of the protonated isopropylidene ribofuranosylamine (V) with (IVb) (prepared by refluxing ethyl-α-amino-α-cyanoacetate with triethyl *ortho*formate in acetonitrile for 45 min) and base, however, gave a mixture of the α- and β-isopropylidene aminoimidazole nucleosides ((VI) and (IIb), respectively), which were very readily separated without chromatography and obtained as crystalline solids (Table I). Closer examination of the earlier reaction of (V) with the methyl ester formimidate (IVa) and base also revealed the presence of the corresponding α-nucleoside.

Table I.

Imidazole nucleoside	Yield (%)	m.p. °C	$[\alpha]_D^{20}$ (C, %)[a]	λ_{max}(nm)[b] [ε]	BRATTON MARSHALL[9] λ_{max}(nm)
(XIV)	75	177–9	−24° (0.4)	266 [12,640]	513
(VI)	22	188–190	−70° (0.3)	267 [11,060]	508
(IIb)	16	180–2	−97° (0.3)	267 [12,900]	512
(IIa)	—	161–2	−100° (0.3)	267 [13,200]	508
(X) [A]	20	225–6	+95° (0.2)	267 [13,000]	518
(XI) [B]	20	190–2	+43° (0.2)	266 [12,700]	508

[a] Measured in DMSO. [b] Slight inflection at 230–240 nm; solvent methanol.

The same mixture of α- and β-nucleosides (VI) and (IIb) was also obtained by a modification of this last synthesis which involved prior reaction of the ribosylamine tosate (V) with ethyl formimidate hydrochloride and triethylamine, and subsequent reaction of the mixture of intermediates (VII), which are presumably formed, with ethyl-α-amino-α-cyanoacetate.

The structure assigned to the methyl β-nucleoside (IIa) was confirmed by elemental analysis, and comparison (TCL, IR, UV, m.p. and mixed m.p.) with material already prepared via the benzoyl intermediate and earlier shown to have the β-configuration by conversion into inosine[2] and into various intermediates in the de novo pathway leading to purine nucleotides.

Assignment of the β-structure to the corresponding ethyl ester nucleoside (IIb) was confirmed by elemental analysis, mass spectrum ($M^+ = 327$) and by comparison of optical rotation (Table I), and circular dichroism (Figure 1) measurements with these of the methyl β-nucleoside (IIa). The β-ethyl ester nucleoside (IIb) with ammonia gave the aminoimidazole carboxyamide (IIc) which was identical (TLC in several solvents) with that produced from the β-methyl ester (IIa). The structure assigned to the ethyl α-ester (VI) was confirmed by elemental analysis, mass spectrum ($M^+ = 327$) and the differences in optical rotation (Table I) and circular dichroism (Figure 2) measurements compared to those of the β-ester (IIb). The α-riboside (VI) also differs from the β-anomer in behaviour on thin layer chromatogram, and rate of loss of isopropylidene group in 10% aqueous acetic acid at 100°C (the β-form required 2 h for complete deacetonation compared with $3^1/_2$ h for the α-form; some aglycone was formed in each case).

In addition the assignments agree well with HUDSON's[4] isorotation rules. These rules have been shown to apply to analogous purine nucleosides[5]. The NMR-spectra were not

[1] J. G. BUCHANAN and S. C. HARTMAN, Adv. Enzymol. *21*, 119 (1959). – S. C. HARTMAN and J. G. BUCHANAN, A. Rev. Biochem. *28*, 365 (1959). – J. BADDILEY and J. G. BUCHANAN, Q. Rev. chem. Soc. *11*, 329 (1957).
[2] G. SHAW and D. V. WILSON, J. chem. Soc. *1962*, 2937.
[3] N. J. CUSACK and G. SHAW, Chem. Commun. *1970*, 1114.
[4] C. S. HUDSON, J. Am. chem. Soc. *31*, 66 (1909).