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DIFFERENCES IN THE CHEMICAL CONSTITUENTS OF *MANGIFERA INDICA*, INFECTED WITH *ASPERGILLUS NIGER* AND *FUSARIUM MONILIFORMAE*

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Key Word Index—*Mangifera indica*; Anacardiaceae; mango; fungal infection; *Aspergillus niger*; *Fusarium moniliformae*; xanthones; mangiferin; 1,3,6,7-tetramethoxyxanthone; 1,3,5,6,7-pentamethoxyxanthone; flavonols; depsides; 6-(*O*-galloyl)-2,4-dihydroxybenzoic acid; 3-*C*-gluco-6-(*O*-galloyl)-2,4-dihydroxybenzoic acid; triterpenes; α-aminoacids; gluco-peptides; cytokinin; toxic peptides; naphtho-γ-pyrones; zearalenone; 12,13-epoxytrichothecenes.

Abstract—The isolation and characterization of the chemical constituents of different parts of *Mangifera indica*, sound and infected with two pathogenic fungi, viz. *Aspergillus niger* and *Fusarium moniliformae*, are described. Natural occurrence of two polyketideshikimate-derived depsides is reported for the first time. Additionally, a number of xanthones, flavonoids, triterpenes and amino acids, not encountered before in this species, are reported. The co-occurrence of mangiferin, 1,3,6,7-tetra- and 1,3,5,6,7-pentaoxygenated xanthones and the quantitative variation of the latter two compounds with the growing of the plant and during the fungal infection are biochemically significant. The protector role of the flavonoids and other C_{15} metabolites to *M. indica* from the ingress of the fungal hyphae is indicated. The two pathogenic fungi secreted a number of mycotoxins in different parts of the host species during its vegetation and flowering periods. During the elaboration of these toxic metabolites, the host–pathogen interaction played an important role. Evidence is presented for *A. niger* as a mycotoxin producing fungus.

INTRODUCTION

Previous investigations of *Mangifera indica* reported the presence of free sugars, phenolic compounds, viz. mangiferin, gallic acid, *m*-digallic acid gallotannin, quercetin, isoquercitrin, kaempferol, astragallin, butin, fisetin, anthocyanins, leucoanthocyanins, mono-, sesqui- and triterpenes in the various parts, e.g. leaves [1–3], fruits [4], flowers [5], bark [6] and in resin [7–9] of this species. But nowhere in these reports was it mentioned whether or not the investigated plant parts were sound or infected, except a casual report about a postinfectional change in the amino acid content of mango fruits caused by *A. niger* [10]. Different parts of *M. indica* are known to suffer from a number of recognized diseases caused by fungi, bacteria, insects and by flowering parasitic plants. Among the diseases, those affecting flowers and fruits are the most destructive ones. Fungi are the major causal agents for the diseases [11, 12]. Since significant chemical changes in host species are known to occur when they are invaded with pathogenic fungi [13], it was thought worthwhile to investigate separately the various sound and infected parts of *M. indica* (cv Banarasi Langra), collected from the orchard of the Banaras Hindu University Campus during January 1976 to April 1977. The details of these findings are described in this paper.

RESULTS AND DISCUSSION

The acetone extractives of healthy leaves, twigs, flowers and fruits of *M. indica* and those of the corresponding parts infected with *A. niger* van Tiegh (CMI-IMI 205879) and *F. moniliformae** Sheld, were separately processed for their various neutral, phenolic, carboxylic and basic constituents. The nature and abundance of the chemical constituents of the individual parts of *M. indica* are recorded in Table 1.

Mangiferin

Different parts of sound and infected mango, at different periods of vegetation, produced varying amounts of mangiferin. The concentration of mangiferin was highest in healthy tender leaves, which gradually declined with maturity of the leaves. Again, among flower-bearing and non-bearing healthy plants, the concentration of magniferin was much higher in tender leaves of the latter. In healthy twigs, mangiferin occurred as a minor entity, while in *malformed* shoots/twigs (collected in November), infected with *F. moniliformae* [11, 12], its concentration was very high. Again, mangiferin content of tender leaves adjoining the malformed shoots, was very low. It would seem likely that mangiferin was translocated into the malformed shoots soon after its synthesis in tender leaves to offer resistance to the fungal attack. This conclusion is consistent with the recently observed anti-*Fusarium* action of mangiferin [14, 15]. In normal flowers, mangiferin was present only in traces, while in *F. moniliformae*-infected malformed flowers (July–August), its amount was very much increased. Malformed flowers, collected during fruit-bearing time (March–April), however, showed a sharp decline in mangiferin content. At this stage, mangiferin was replaced by depsides (C_6–C_7) and polyphenols (C_{15} moieties). The increased production of the latter could be interpreted as a counteraction to the ingress of the fungal hyphae into the tracheids of the host plant. Polyphenols are known to be potent enzyme

* The microflora has been deposited to the Commonwealth Mycological Institute, Kew, Surrey, England, for confirmation and strain number.

Table 1. Yield and composition of the total acetone extractives of different parts of sound and infected *M. indica**

Plant parts	Total extractives percent yield	Xanthones	Flavonols	Depsides	Free phenols	Triterpenes	Polyphenols	Unidentified compounds
Fruits								
Healthy	10	—	1	—	0.5	1	6	1.5
Infected†	14	—	1.5	0.5	2	1	8	1
Leaves								
Healthy (tender)‡	12	5	1	—	—	1	4	1
Healthy (tender)§	16	8	1	—	—	1	5	1
Infected (tender)‖	12	1.5	2	—	—	1	7	0.5
Healthy (mature)	10	1	3	—	—	1	4	1
Flowers								
Healthy	20	1	—	—	—	2	12	5
Malformed¶, **	24	8	—	0.5	—	1	10	4.5
Malformed¶, ‡‡	20	1	—	0.5	—	1	12	5.5
Infected††, ‡‡	20	1	2	3	—	2	10	2
Twigs (shoots)								
Healthy	12	1	1	—	—	3	6	1
Malformed¶, §§	20	6	2	1.5	—	3	6	1.5
Malformed¶, ‡‡	23	2	2	0.5	—	3	12	3.5

* The plant materials were collected three times each during the periods mentioned; mean of three sets of reading for each experiment has been recorded. † *A. niger*; ‡ from flower-bearing plants; § from non-bearing plants; ‖ adjoining malformed shoots; ¶ *F. moniliformae*; ** collected in July–August; †† *F. moniliformae* spores (secondary infection); ‡‡ collected in March–April; §§ collected in November; — denotes absent.

precipitants and can inhibit the extra-cellular hydrolases secreted by fungal pathogens [13].

Contrary to a previous report [4], no trace of mangiferin was detected in either healthy or in *A. niger*-infected fruits. Instead, a number of free phenols, viz. phloroglucinol, 1,2,3,4- and 1,2,3,5-tetrahydroxy benzenes, were isolated from the above parts.

1,3,6,7-Tetraoxygenated xanthone

This compound was isolated, as its perMe ether, in small quantities, from healthy twigs and flowers of *M. indica*. Its concentration was considerably increased when these parts were infected with *A. niger* and *F. moniliformae*. It is of note that the complementary pair (1,3,5,6-) of this xanthone was absent in this species. Further, the 1,3,6,7-tetraoxygenated xanthone was only present when mangiferin was present in appreciable quantities. Its genesis would therefore seem to be related with the elaboration of mangiferin. 1,3,6,7-Tetraoxygenated xanthone has not been reported before in *M. indica*.

1,3,5,6,7-Pentaoxygenated xanthone

This compound was also isolated as the perMe ether along with the tetraoxygenated xanthone. The relative amounts of these two xanthones varied at different stages of the *F. moniliformae* infection. Thus, while there was a slow decline in the concentration of the tetraoxygenated xanthone in infected flowers, the pentaoxygenated xanthone concomittantly was accumulated. In the context of increased polyphenol oxidase activity in host plants on fungal infection [13], these observations would seem to suggest that the tetraoxygenated xanthone is a likely precursor of the pentaoxygenated xanthone in *M. indica*. The natural occurrence of 1,3,5,6,7-pentaoxygenated xanthones has been reported only once before in *Canscora decussata* [16].

Kaempferol, quercetin and myricetin

During the fruiting and subsequent resting periods (May and November), the 3 flavonols were isolated from healthy and *A. niger* infected leaves and fruits, and *F. moniliformae* infected shoots of *M. indica*. The flavonols were absent in malformed (sterile) flowers during these periods. They, however, appear in malformed flowers during normal flowering time (February–March). At this stage, healthy flowers were free from flavonols, but flowers invaded with spores of *F. moniliformae* (which we termed 'secondary infection') contained these flavonols. The postinfectional formation of the flavonols in flowers of *M. indica* suggests that they might function as phytoalexins [17].

In healthy leaves, the concentration of the 3 flavonols increased with maturity. Further, in 3 to 4 week-old leaves, the corresponding 3- and/or 5-*O*-glycosides (rhamnoside, glucoside) appeared. These observations would seem to suggest that the flavonols act as binders of carbohydrates which are essential for blossoming.

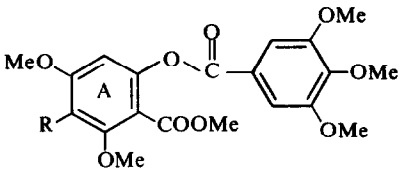
Depside of phloroglucinol carboxylic acid and gallic acid (**1**)

Malformed flowers (November) and flowers with secondary infection (March) contained this compound in appreciable quantity. It was isolated as the Me ether ester and its structure was established as Me-6-(*O*-triMe-galloyl)-2,4-dimethoxybenzoate (**1**) on the basis of chemical and spectral evidence (see Experimental).

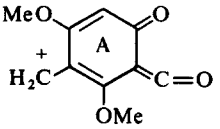
C-Gluco-depside (**2**)

This compound co-occurred with **1**, in malformed flowers, as a minor entity and was also isolated as the Me ether ester. It showed a striking similarity to **1** in its UV spectrum. It did not give any observable M^+ in its MS. Significant fragment ions, however, appeared due to tri-*O*-Me galloyl and Me-*C*-hexosyl-*O*-diMephloroglucinol carboxylate moieties. The latter ion further fragmented to give fragment-A. The presence of the *C*-hexosyl

function was further suggested from its resistance to furnish any hexose on acid hydrolysis. Alkali hydrolysis afforded 3,4,5-trimethoxybenzoic acid and another compound that responded to the benzidine–metaperiodate test for sugars. The latter compound was chromatographically identical with a synthetic *C*-glucosyl-phloroglucinol. The formation of the *C*-glucosylphloroglucinol entity from the corresponding *C*-glucophenolic carboxylic acid presumably had taken place during acid treatment of the alkali hydrolysed product of the glucodepside. The glucodepside is assigned structure **2** on the basis of spectral and chemical evidence (see Experimental).



1: R = H
2: R = Glucosyl



Fragment A (*m/e* 193)

The two depsides (**1** and **2**) are unique since they are clearly polyketideshikimate-derived compounds. The only other naturally occurring depsides, encountered before in lichens, were polyketide (acetate units)-derived compounds. The two aryl moieties present in **1** and **2** bear close resemblance to those of mangiferin. In the context of the generally accepted role of mangiferin as a 'switching' chemical character in the evolutionary series of flavonoids [18], a C_6–C_3–C_6 entity could be visualized as the common intermediate enroute to **1**, **2** and mangiferin in *M. indica*. Thus, acyl migration in **2**, followed by decarboxylation and ring closure of the resulting benzophenone would complete the process of mangiferin biosynthesis. Alternatively, the depside formation in *M. indica* could be due to the stress condition developed in the host species upon the fungal infection. Degradation of flavonoids into depsides *in vitro*, by use of fungal mycelium, is a well documented process [19].

Polyphenols

Mango leaves, twigs and flowers yielded catechin, gallocatechin and cyanidin in accordance with the previous reports [5, 6] of their occurrence in the various parts of *M. indica*. During the *Fusarium* infection, these compounds were, however, largely replaced by some polymeric compounds which we termed polyphenols (Table 1). Hydrolysis of perMe ethers of these compounds afforded tri-*O*-Megallic-, di-*O*-Meprotocatechuic- and tri-*O*-Mephloroglucinol carboxylic acids and glucose. These were presumably the breakdown products of some depsides since the spectral properties of the mixture of polyphenols suggested the presence of aryl esters and carboxylate moieties. Another interesting observation was the seasonal variation in the amounts of polyphenols in malformed shoots and flowers. The concentration of these compounds was highest in the infected parts during the normal fruiting season (April–May) when the spore concentrations of the two pathogenic fungi, *A. niger* and *F. moniliformae*, were also very high in the orchard of the Banaras Hindu University Campus. The increased production of the polyphenols by *M. indica* is consistent with the reported protector role of such compounds against fungal infection [13, 18].

Gallic and protocatechuic acids

These phenolic acids were isolated from fruits and leaves of mango only when these parts were infected with *A. niger*. Surface-sterilized healthy fruits inoculated with the fungus and incubated for about a week at 21°, also produced these two phenolic acids in appreciable quantities. The two acids were not normal metabolites of mango since they were absent in healthy leaves, twigs, flowers and green fruits.

Triterpenes

Four triterpenes, viz. lupenone, lupeol, ursolic acid and glochidonol, were isolated from the different parts of mango. These triterpenes have not been reported before in this species. The fungal infections did not alter the production of these triterpenes either qualitatively or quantitatively. It is, however, interesting to note that the nature of the triterpenes varied with the variety of mango. Thus, the triterpenes reported before for other varieties of mango [7–9] were different from those isolated from the 'Banarasi Langra'.

Amino acids

Contrary to a previous report [10], the amino acid contents of healthy and *A. niger*-infected green fruits were found to be qualitatively very similar. Six common protein amino acids were detected in these tissues. In *A. niger*-infected fruits, however, there was a considerable decline in the quantity of these amino acids. This is presumably due to their transformation into polypeptides, e.g. of the malformin [20] and tremorgen [21] types. While the production of these two types of toxic polypeptides by the members of the *Aspergillus* is a well documented phenomenon [21], the present investigation has demonstrated for the first time the role of host–pathogen interaction in the formation and abundance of these toxins. When *A. niger* (IMI-205879) was cultured in Richard's medium in the presence of autoclaved mango, the CNS depressant peptides [14] of the malformins type and the tremor producing toxins [14] of the tremorgenic types were produced only in traces. Fresh mango fruits infected with this pathogen produced both the toxins in very high yields. This phenomenon is a cause for alarm from a public health view point. Diseased mango fruits are often used by unscrupulous traders for the preparation of processed mangoes, e.g. pickles, jams, chutney, etc., and thereby provide high toxin risk in man.

From the *F. moniliformae*-infected twigs and shoots, a cytokinin factor was isolated at an early stage of the infection. It showed spectral and biological properties characteristic of purine derivatives. This compound was absent in healthy twigs. A closely related compound was isolated from culture filtrates of *F. oxysporum* f.sp. *carthami* when the fungus was grown in Richard's medium [15]. The cytokinin factor was metabolized at the terminal stage of the infection. The infected parts at this stage turned black and contained mainly polymeric quinones.

Gluco-peptides

Leaves (of both flower-bearing and non-bearing plants) and flowers of *M. indica* plants (*ca* 35–40%), in the orchard of the Banaras Hindu University Campus, were found to exude a sweet sticky substance when the spore concentrations of *F. moniliformae* and *A. niger* were very high (February–March, 1977). Mango hopper (*Idiocerus* species), a pest of mango, was also partially responsible for the drain of the sap. The exudate was freely soluble in water. From the aqueous extractives, fructose, amino-cyclitols, a mixture of complex nitrogenous entities, and mangiferin-*O*-glucoside were separated through their acetates and perMe ethers. Acid hydrolysis of perMe ethers follows by column chromatography afforded tri-*O*-Me gallic acid and phloroglucinol triMe ether as the major non-nitrogenous entities. These compounds presumably resulted from depside(s) of the type **1**. PLC of the mixture of acetates followed by acid hydrolysis yielded mangiferin (in traces), glucose, and three amino acids, viz. proline, alanine and aspartic acid. In the process of exudation, the essential tree reserves were considerably depleted and the affected flower-bearing plants remained unproductive. Soon after the normal flowering period, these showed aggravated symptoms of the malformation.

Naphtho-γ-pyrones

Different strains of *A. niger* were reported to produce a variety of acetogenins among which flavasperone and aurasperone [22] deserve special mention due to their significant biological properties [23]. The mycelial extract of *A. niger* (IMI-205879) afforded 5 naphtho-γ-pyrones, viz. flavasperone, rubrofusarin, aurasperone-A, aurasperone-A monohydrate and aurasperone-D. These yellow pigments were also present in *A. niger*-infected mango fruits. The extractives of the infected mango fruits, containing the naphtho-γ-pyrones, when administered to laboratory animals produced strong toxic symptoms leading to death by respiratory arrest [14].

Zearalenone and 12,13-epoxy-trichothecenes

Three recognized mycotoxins, viz. zearalenone, diacetoxyscirpenol and T-2 toxin, were isolated from malformed flowers and shoots of *M. indica* infected with *F. moniliformae* [14]. Since extractives of these parts of mango are liberally used in indigenous system of medicine for a variety of purposes [24], particular care should be taken about the soundness of plants at the time of collection. While the elaboration of mycotoxins by a wide range of strains of *F. moniliformae* is well documented [25], the bio-production of mycotoxins by *A. niger* was reported only once before in mould-damaged rice [20]. On the basis of the present report of elaboration of toxic peptides by another strain of *A. niger*, the mould should hereafter be considered as one of the mycotoxin producing fungi.

EXPERIMENTAL

All mps were taken on a Kofler block in open capillaries and are uncorr. UV spectra were recorded in MeOH unless otherwise stated and IR spectra were determined in Nujol and only the major bands are quoted. MS were determined at 70 eV. PMR spectra were obtained at 60 MHz using TMS as an int. stand. Separation by column chromatography was carried out using Si gel (BDH, 60–120 mesh) and TLC experiments were conducted with Si gel G. Six solvent systems, viz. C_6H_6–HOAc (50:1, solvent 1), C_6H_6–HOAc (25:1, solvent 2), $CHCl_3$–HOAc (50:1, solvent 3), $CHCl_3$–MeOH (20:1, solvent 4), $CHCl_3$–MeOH–HOAc (10:1:1, solvent 5) and *n*-BuOH–HOAc–H_2O (4:1:2, solvent 6), were used as developers. I_2, $FeCl_3$, ninhydrin, and Liebermann–Burchard reagents were used for visualization.

General extraction procedure. In a typical expt, malformed mango shoots (5 g), infected with *F. moniliformae*, were macerated with Me_2CO in a high-speed blender. After 4 hr, the mixture was filtered and the solvent removed under red. pres. The extractives were poured into H_2O (100 ml) and the suspension was successively extracted with Et_2O (fraction A) and EtOAc (fraction B) (50 × 3 ml portions each). At the interface of the aq. EtOAc extract, mangiferin (83 mg) separated and was collected by filtration. The identity of mangiferin was established by direct comparison (mp, mmp, co-TLC, IR) with an authentic sample [26]. The H_2O mother liquor (fraction C) was retained.

Treatment of fraction A. The residue (0.15 g) from this fraction on trituration with EtOH afforded a colourless solid (62 mg), mp 225–230°. Analytical TLC (solvent 1) showed the presence of 3 triterpenes. These were separated by column chromatography on Si gel (14 × 2 cm) using C_6H_6 (2 l.) as eluent and 200 ml fractions were collected. *Lupenone.* The residue from fractions 2 and 3 crystallized from EtOH as colourless needles (12 mg). The identity of this compound was established by direct comparison (mp, mmp, IR) with an authentic sample. *Lupeol.* Fractions 5–6 on re-chromatography afforded lupeol (43 mg) (mp, mmp, acetate). *Glochidonol.* Fractions 8–9 on re-chromatography, followed by PLC gave glochidonol (5 mg). The identity of this compound was established by comparison (mp, $[\alpha]_D$, MS of the acetate) with lit. [27]. *Ursolic acid.* Treatment of fraction A from healthy green mango pulp (75 g) and tender leaves (9 g) yielded ursolic acid (25 mg) and (8 mg), respectively, in addition to the 3 triterpenes mentioned above. Its identity was established by direct comparison (mp, mmp, IR of Me ester acetate) with an authentic sample.

Treatment of fraction B. Attempts to separate the constituents of this fraction by column or layer chromatography failed. The mixture (0.4 g) was therefore methylated with $(Me)_2SO_4$–K_2CO_3–Me_2CO under reflux (46 hr). Work up in the usual fashion followed by PLC (solvent 3) gave the following compounds. *1,3,5,6,7-Pentamethoxyxanthone.* The uppermost blue fluorescent zone, R_f 0.5, was eluted with $CHCl_3$. On evapn of the solvent, the xanthone was obtained as colourless crystals (6 mg). The identity of the xanthone was established by direct comparison (mp, mmp, co-TLC, UV, MS) with an authentic sample [16]. *1,3,6,7-Tetramethoxyxanthone.* The middle blue fluorescent zone, R_f 0.4, afforded this xanthone as pale yellow crystals (5 mg). The identity of the compound was established in the usual way (mp, mmp, co-TLC, UV) [28]. *Kaempferol, quercetin, myricetin (as Me ethers).* The lowest sea-green fluorescent zone, R_f 0.15, was eluted with $CHCl_3$. On evapn of the solvent, the perMe ethers of the 3 flavonols were obtained as a mixture (12 mg). Repeated PLC of the mixture (solvent 3) afforded the perMe ethers as pure entities. The identity of these compounds was established in the usual way (mp, mmp, co-TLC, UV) [29]. *Quercitrin Me ether.* The mixture of perMe ethers from the EtOAc extractives of mature leaves showed the presence of an additional olive-green fluorescent zone at the base line. It was separated by re-PLC (solvent 4) as a homogeneous gum (9 mg), R_f 0.5; UV: λ_{max} 252, 260–267 *sh*, 317, 360 nm. On acid hydrolysis (4% HCl), it furnished 5-hydroxy-3,7,3',4'-tetramethoxyflavone (mp, mmp, Co-TLC, UV) and glucose (PPC). *Isoquercitrin Me ether.* Proceeding as above isoquercitrin methyl ether (8 mg) was obtained from mango shoots infected with *F. moniliformae*.

Me-6-(O-triMegalloyl)-2,4-dimethoxybenzoate (**1**). A portion (0.1 g) of the EtOAc extractives of malformed flowers was permethylated with $(Me)_2SO_4$–K_2CO_3–Me_2CO under reflux 46 hr). The mixture of perMe ethers was subjected to column chromatography (14 × 1.8 cm). Elution was carried out with C_6H_6 and different proportions of C_6H_6–EtOAc. The C_6H_6–EtOAc (10:1) eluates afforded a brown solid which crystallized from EtOH as colourless microcrystals (25 mg), mp 125°, R_f 0.7 (solvent 2); UV: λ_{max} nm (log ε) 256 (3.85) and 340–344 (3.52);

PMR: δ ($CDCl_3$) 7.48 (1H, *d*, *J* = 2 Hz), 7.43 (1H, *d*, *J* = 2 Hz), 7.39 (1H, *d*, *J* = 2.5 Hz), 7.2 (1H, *d*, *J* = 2.5 Hz) (aromatic 4H), 3.9–3.95 (15H, OMe), 3.88 (3H, $-CO_2Me$); MS: *m/e* 406 (M^+, relative intensity, 21%), 375 (1), 212 (40), 211 (2), 197 (62), 195 (100), 181 (8), 167 (8), 152 (12), 151 (7), 149 (1), 137 (10), 133 (2). (Found: C, 58.83; H, 5.62. $C_{20}H_{22}O_9$ requires C, 59.1; H, 5.4%). On hydrolysis with aq. KOH (5%) at room temp. for 2 days, **1** (25 mg) afforded gallic acid tri-*O*-Me ether (7 mg) (mp, mmp, IR) and 2-hydroxy-4,6-dimethoxybenzoic acid (12 mg) (mp, mmp, IR).

Synthesis of the depside **1**. *2,6-Dihydroxy-4-methoxy-methylbenzoate*. 2,4,6-Trihydroxybenzoic acid (0.2 g) was refluxed with Me_2SO_4 (1 ml) and $NaHCO_3$ (1 g) in Me_2CO (50 ml) at 100° for 4 hr. Work up gave Me-2,6-dihydroxy-4-methyoxbenzoate as the major product (0.17 g), R_f 0.4 (solvent 1), along with other products (7 mg). The title compound was separated by PLC. The lower light yellow zone afforded Me-2,6-dihydroxy-4-methoxybenzoate as colourless crystals (0.15 g), mp 95–98°; MS: *m/e* 198 (M^+), 183, 167, 152. *Me-6-(O-trimethylgalloyl)-2-hydroxy-4-methoxybenzoate*. The above solid (0.1 g) in Et_2O (5 ml) was condensed with 3,4,5-trimethoxybenzoyl chloride (0.116 g) in Et_2O (5 ml) in the presence of C_5H_5N (0.2 ml) at room temp. with occasional shaking (24 hr). The solvent was removed, the residue redissolved in Et_2O (40 ml) and filtered through a column of Al_2O_3 (10 g). The filtrate on evapn gave a solid (0.18 g) which crystallized from EtOH as colourless microcrystals, mp 150–155°, R_f 0.62 (solvent 2). Methylation with $(Me)_2SO_4$–K_2CO_3–Me_2CO under reflux (40 hr) afforded the depside **1** as colourless solid, mp 125°; mmp, co-TLC, IR, and MS spectra of the synthetic sample and the natural product were identical.

3-C-Gluco-Me-6-(O-triMegalloy)-2,4-dimethoxybenzoate (**2**). The EtOH mother liquor, after separation of **1**, afforded an amorphous solid (9 mg), R_f 0.51 (solvent 2); $[\alpha]_D^{24}$ +15° (*c* 0.38, MeOH), λ_{max} nm (log ε) 256 (3.78), 340–344 (3.50); MS: *m/e* 374 (rel. intensity, 4%), 373 (10, methyl-*O*-di-Me-*C*-glucosyl-phloroglucinol carboxylate moiety), 315 (80, *O*-di-Me-*C*-glucosyl-phloro glucinol moiety), 297 (10, *O*-Me-*C*-glucosyl-phloroglucinol moiety $-H_2O$), 211 (80, *O*-triMegallate), 915 (100), 193 (14, fragment-A), 167 (8), 165 (7), 44 (22). (Found: C, 54.5; H, 5.4. $C_{26}H_{32}O_{14}$ requires C, 54.9; H, 5.6%).

Cytokinin. Fraction C was acidified with dil. HCl when a tan-coloured solid was pptd. The solid was filtered off and the aq. acidic layer cooled and basified with NH_4OH when a brown solid was pptd. The latter crystallized from dioxan as pale brown crystals which softened at 225° and decomposed above 350°. It responded to the N fusion test; R_f 0.48 (solvent 6); brownish-yellow with ninhydrin reagent; UV: $\lambda_{max}^{MeOH-HCl}$ 260, 265–267, 340–355 *sh*; IR: ν_{max}^{KBr} 3200–3400 (*br*), 2350, 1755, 1680, 1642, 1100, 1040 cm^{-1}. The UV and IR data indicate the presence of a purine nucleus [30]. The vegetative growth-promoting property of this compound was tested, as described before [15], and found to be significant.

Extraction of flower and leaf-exudates. Flower and leaf-exudates were separately dissolved in H_2O and filtered. The solvent was evapd from the filtrates when a pale brown gum was obtained in each case. PPC (solvent 6) of the two extractives indicated that there was no qualitative variation in the constituents. Further, fructose and two strongly polar constituents were found to be the major entities. Attempts to separate these latter constituents by column or layer chromatography were unsuccessful. A portion of the gum (0.2 g), from the flower exudates, was acetylated with Ac_2O (6 ml) and C_5H_5N (0.4 ml) under reflux (4 hr). The mixture of acetates was subjected to PLC (solvent 6) when two major bands were separated. These were eluted with $CHCl_3$–MeOH (1:1). *Amino cyclitols* (as acetates). The upper pale yellow zone, R_f 0.7, responded to colour tests for *N*-acetyl cyclitols. The IR: ν_{max}^{Nujol} 1645 cm^{-1} (*br*) also suggested the presence of a N–CO grouping. Hydrolysis with aq. HCl (4%) afforded the parent amino cyclitol(s) which responded to tests for both sugars and amino groups [31]. *Acetates of gluco-peptides*. The lower brown zone, R_f 0.2, was also subjected to acid hydrolysis. Working up afforded glucose (PPC, acetate), mangiferin (PPC, TLC, UV) and 3 amino acids, viz. proline, alanine and aspartic acid (PPC, TLC).

Permethylation of the flower-exudate. A portion of the brown gum (0.2 g) was methylated with $(Me)_2SO_4$–K_2CO_3–Me_2CO under reflux (46 hr). After work up, a portion (0.05 g) was hydrolysed with aq. HCl (4N). The product was extracted with $CHCl_3$. The aq. layer showed the presence of glucose (PPC). The organic layer was concd and column chromatographed (10 × 0.5 cm). Elution was carried out with C_6H_6 (1 l.) and fractions (50 ml) were collected. The earlier C_6H_6 eluates were combined and evapd to give a light yellow oil (0.015 g). The oil was hydrolysed (MeOH–HCl, 10%) and the product was re-chromatographed when tri-*O*-Megallic acid (4 mg) and 1,3,5-trimethoxybenzene (2 mg) were obtained. The identity of the two compounds was established by mmp, co-TLC, and IR.

Amino acids from polypeptides. The residue (4.5 g), from the EtOAc extract of mycelium (18 g) of *A. niger*, was successively triturated with petrol, C_6H_6 and $CHCl_3$ to remove the less polar yellow pigments. The insoluble residue (2.8 g) was hydrolysed with HCl (6N) at 100°. The acidic hydrolysate showed the presence of 5 amino acids (PPC, TLC) as the major compounds. The mixture of amino acids was subjected to gradient elution from a column of Amberlite CG-120. The individual acids were characterized by direct comparison (PPC, co-TLC) with authentic amino acids as aspartic acid, proline, valine, and cysteine.

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REFERENCES

1. Gabry, Y. M. E., Osman, A. M. and Sheta, A. E. (1973) *Indian J. Chem.* **11**, 405.
2. Sissi, E., Hassan, I. and Saleh, N. A. M. (1970) *Planta Med.* **18**, 73.
3. Paris, R. R. and Jacquemin, H. (1970) *Compt. Rend.* **270**, 3232.
4. Ansari, M. A. E., Reddy, K. K., Sharma, S. K. N. and Nayudamma, Y. (1969) *Leather Sci.* **16**, 13.
5. Bose, J. L. and Siddiqui, S. (1948), *J. Sci. Ind. Res. (India)* **7B**, 100.
6. Ansari, M. A. E., Rajadurai, S. and Nayudamma, Y. (1967) *Leather Sci.* **14**, 247.
7. Corsano, S. and Mincione, E. (1965) *Tetrahedron Letters* 2377.
8. Corsano, S. and Mincione, E. (1967) *Ann. Chim. (Rome)* **57**, 522.
9. Corsano, S. and Mincione, E. (1967) *Ann. Chim. (Rome)* **57**, 508.
10. Singh, R. H. and Tandon, R. N. (1970) *Phyton* **27**, 101.
11. Roychaudhuri, S. P. and Pathak, S. P. (1966) *Indian Phytopathol.* **19**, 227.
12. Sharma, O. P. and Tiwari, A. (1975) *Pesticides* **9**, 44.
13. Smith, I. M. (1971) in *Fungal Pathogenicity and the Plants Response*, (Byrde, R. J. W. and Cutting, C. V. eds) pp. 355–364. Academic Press, New York.
14. Ghosal, S., Biswas, K., Chakrabarti, D. K., Bhattacharya, S. K. and Chattopadhyay, B. K. (1977) Abstracts 2 Conference Commonwealth Pharm. Assoc., Bombay, p. B-2.
15. Ghosal, S., Biswas, K., Chakrabarti, D. K. and Basuchaudhary, K. C. (1977) *Phytopathol.* **67**, 548.
16. Ghosal, S., Chaudhuri, R. K. and Markham, K. R. (1974) *J. Chem. Soc. Perkin 1* 2538.
17. McClure, J. W. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds) pp. 970–1055. Chapman & Hall, London.

18. Swain, T. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds) pp. 1097–1129. Chapman & Hall, London.
19. Barz, W. and Hösel, W. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, J. eds) pp. 916–969. Chapman & Hall, London.
20. Anderegg, R. J., Biemann, K., Buichi, G. and Chuohman, M. (1976) *J. Am. Chem. Soc.* **98**, 3365.
21. Yamazaki, M., Sasage, K. and Miyaki, K. (1974) *Chem. Commun.* 408.
22. Tanaka, H., Wang, P. L., Yamada, O. and Tamura, T. (1966) *Agr. Biol. Chem.* **30**, 107.
23. Biswas, K. and Ghosal, S. unpublished work.
24. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) in *Glossary of Indian Medicinal Plants* p. 161. Council of Scientific and Industrial Research, New Delhi.
25. Mirocha, C. J. and Christensen, C. M. (1974) *Ann. Rev. Phytopathol.* **12**, 303.
26. Chaudhuri, R. K. and Ghosal, S. (1971) *Phytochemistry* **10**, 2425.
27. Hui, W. H. and Fung, M. L. (1969) *J. Chem. Soc.* (C) 1710.
28. Ghosal, S. and Chaudhuri, R. K. (1973) *Phytochemistry* **12**, 2035.
29. Ghosal, S., Chauhan, R. B. P. S. and Srivastava, R. S. (1974) *Biochem. J. (India)* **1**, 64.
30. Bentley, H. R., Cunningham, K. G. and Spring, F. S. (1951) *J. Chem. Soc.* 2301.
31. Patridge, S. M. (1948) *Biochem. J.* **42**, 238.