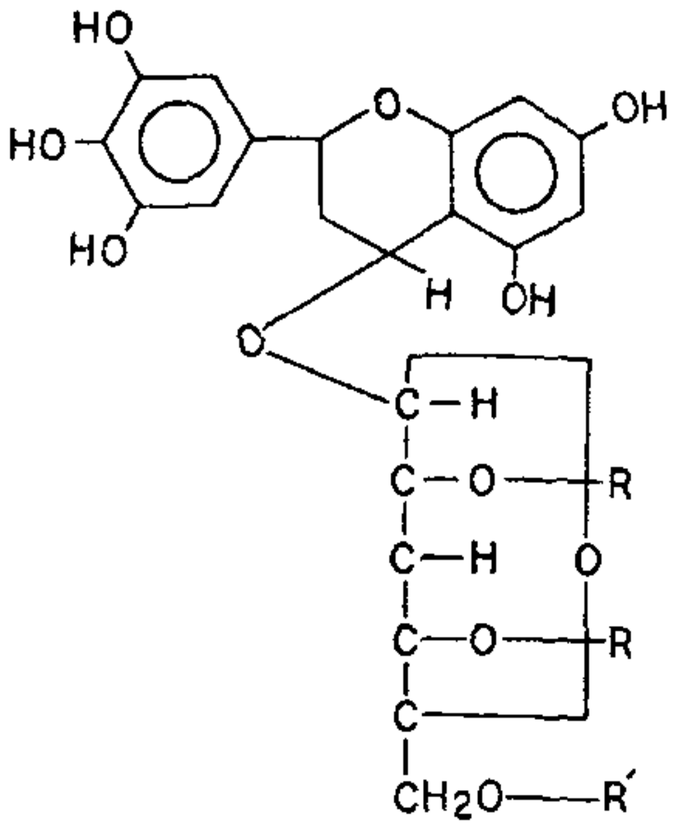
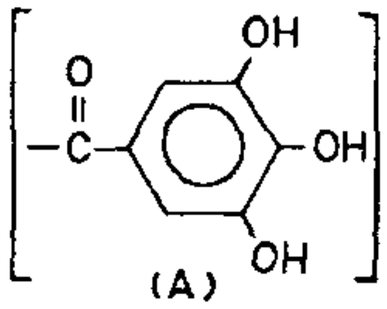
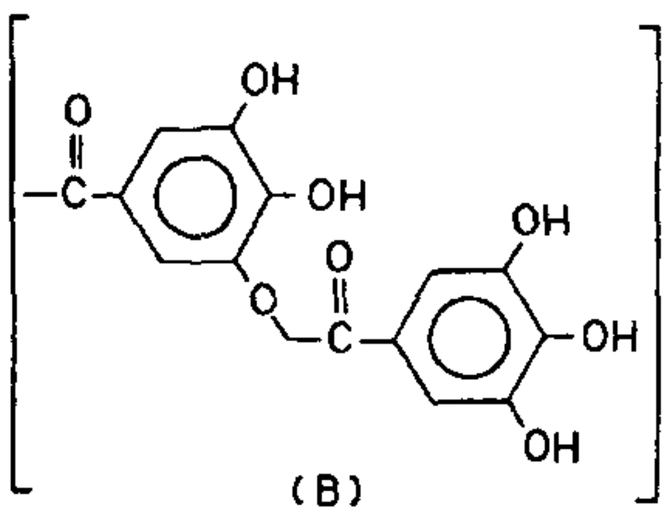




(A)

II (A) R = R′ = A
(B) R = A, R′ = B



(B)

presence of trigalloyl or tetragalloyl chain was excluded. It was, therefore, obvious that only one *m*-digalloyl unit is present in the molecule. The exact position of the *m*-digalloyl unit on glucose core, could not be decided, distribution of three galloyl and one *m*-digalloyl units being random. A tentative structure (IIB) could be assigned to the tannin, based on the fact that in majority of the naturally occurring gallotannins the polygalloylated chain is consistently present at C-6, primary hydroxyl of glucose. This position is also sterically favourable. Spectral data (UV, IR) also confirm the assigned structure.

30 March 1983; Revised 17 December 1983

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STUDIES ON RING OPENING OF COUMARINS

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VARIOUS substituted O-methoxy cinnamic acids have been prepared by ring opening of coumarins using sodium hydride and methyl iodide in dry tetrahydrofuran[1]. The use of sodium hydride-methyl iodide combination was justified by stating that alkaline hydrolysis of coumarins in the presence of dimethyl sulphate yielded predominantly trans-O-methoxy cinnamic acids[2]. However, a careful analysis of the report[2] reveals that *cis*-isomer was obtained exclusively and not a mixture with trans-predominance as understood by Sehgal *et al*[1]. In view of this misrepresentation in the literature, it was considered worthwhile to reinvestigate this reaction with particular emphasis on the examination of the effect of changing the methylating agent from methyl iodide to dimethyl sulphate and also to understand the role of sodium hydride in the ring opening of coumarins.

The starting material could only be recovered when the reaction was carried out under identical conditions[1]. In view of this inability to reproduce the results, the reaction was carried out under various experimental conditions. The observations are summarised in table 1.

It is seen from table 1 that no change could however be effected by the use of sodium hydride dispersed in oil or pure sodium hydride or aqueous sodium hydroxide. The reaction could not also be effected in an inert atmosphere. Substituted *cis*-O-methoxy methyl cinnamates were however obtained when the reaction was carried out at room temperature with many-fold excess of methyl iodide. Hence an alternate methylating agent, namely dimethyl sulphate, was employed with a

Table 1 *Reactions of coumarins with methyl iodide (4 equiv) in dry THF with NaH/NaOH (2 equiv)*[a]

Reactants	Reaction conditions	Observations
Coumarin + NaH	RT, 24 hr	[b]
Coumarin + NaH	reflux, 12 hr	[b]
Coumarin + NaH (1 equiv)	reflux, 12 hr	[b]
Coumarin + NaH[c]	RT, 12 hr	Cis-O-methoxy-methyl cinnamate (20%)
Coumarin + 50% aq. NaOH	reflux, 24 hr	[b]
7-Methoxy-4-methyl coumarin + NaH	RT, 24 hr	[b]
7-Methoxy-4-methyl coumarin + NaH	reflux, 12 hr	[b]
7-Methoxy-4-methyl coumarin + NaH[c]	RT, 24 hr	methyl(2,4-di-methoxy-β-methyl) cinnamate (16%) (*Z*)
7-Methoxy-4-methyl coumarin + 50% aq. NaOH	reflux, 24 hr	[b]

[a] The reactions were also carried out under nitrogen atmosphere; Reactions were carried out with NaH, either dispersed in oil or in pure condition.
[b] starting material recovered;
[c] 8 equiv. of methyl iodide was used;

Table 2 *Reactions of coumarin with dimethyl sulphate (2 equiv) sodium hydride (2 equiv)*

Substrate	Product	Yield (%)
Coumarin	Methyl cis-O-methoxy cinnamate	25
7-Methoxy-4-methyl coumarin	Methyl (2,4-dimethoxy-β-methyl) cinnamate (*Z*)	22
7-Benzyloxy-4-methyl coumarin	Methyl(4-benzyloxy-2-methoxy-8-methyl) cinnamate (*Z*)	18
7-Allyloxy-4-methyl coumarin	Methyl(4-allyloxy-2-methoxy-β-methyl) cinnamate (*Z*)	12
4,7-Dimethyl coumarin	Methyl (4-methyl-2-methoxy-β-methyl) cinnamate (*Z*)	20

view to finding out its efficiency as well as to avoid the use of excess methyl iodide. The results obtained are given in table 2.

The necessity of the base, sodium hydride for effecting the reaction is established on the basis of the following observations.

1. Coumarins did not undergo any change when refluxed in THF with methyl iodide or dimethyl sulphate.
2. The use of weak bases like potassium carbonate or sodium carbonate in THF could not also bring about the ring opening.
3. The reaction does not proceed with a strong base like aqueous sodium hydroxide in THF even under reflux conditions (65°C).

Further, the observation that the ring opening does not take place even with sodium iodide and methyl iodide rules out the possibility that the reaction could have taken place as a result of interaction between methyl iodide and sodium hydride.

It must however be stated that though the present investigation could unequivocally establish the necessity of the use of strong base like NaH, its actual role in effecting the reaction could not be established beyond doubt.

In a typical experiment, a mixture of the coumarin (2.05 mmol, 300 mg), NaH dispersed in oil (4 mmol, 200 mg) and methyl iodide (15.8 mmol, 2.25 g) in dry THF (15 ml) was stirred at room temperature for 24 hr. The solvent was evaporated, diluted with ice-cold water and extracted with chloroform. The chloroform layer was then washed with water, dried and chromatographed over silica gel using chloroform as the eluent. In all the cases a gummy product was obtained. The aqueous layer on acidification did not yield the expected acid. The reaction mixture was refluxed in THF for 24 hr when dimethyl sulphate was used. The products were characterised by ^{1}H-NMR spectra.

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CORRELATION OF NATURALLY OCCURRING CONCENTRATIONS OF FREE-AMMONIA IN THE CAECUM OF RATS WITH RESISTANCE TO EXPERIMENTAL *ENTAMOEBA HISTOLYTICA* INFECTION

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YOUNG rats are generally used as experimental hosts for *Entamoeba histolytica* infection in virulence and chemotherapeutic studies. Infection is induced according to Jones[1] by directly inoculating the amoebae into the caecum at the time of laparotomy. The development of caecal amoebiasis of each infected rat is graded by criteria described in literature[1–3]. The average degree of infection (ADI) provides the virulence status of the parasite. A strain of *E. histolytica* is considered virulent if it can cause caecal mucosal ulceration.

It has often been recorded that despite similar test procedures, differential development of *E. histolytica* infection occurs and in some rats, the amoebae fail altogether to colonize. The factors that have received serious attention in the experimental production of *E. histolytica* infection include the quality of amoebic inocula, bacterial factors and dietary status of the host[4].

In earlier studies[5,6], the caecum of rats with faecal pH 5.5–6.5 was found to be susceptible to *E. histolytica* infection and not those with pH 7.5. In this communication the above results are further substantiated and the death of these amoebae in the caecum of rats bearing alkaline contents correlated with the naturally occurring concentration of free ammonia.

Two strains of *E. histolytica*, B-1 and NIH-200 were used in this study. The former was freshly isolated from an acute case of human amoebiasis and maintained under xenic condition in modified Boeck and Drabohlav medium[7]. The latter was maintained axenically in modified TPS-1 medium[8].

Weanling albino rats (21-day old) (Druckrey), weighing 18–20 g, were obtained from the stock colony maintained at the Animal House of the Institute. Only those rats with faecal pH 5.5–6.5 or 7.5 were used for inoculation and the rest were discarded. The procedures used in determining the faecal and caecal pH and also in the production and evaluation of caecal amoebiasis of rats have been reported earlier[5].

The results (table 1) show cent per cent development of caecal amoebiasis in animals possessing faecal pH 5.5–6.5. In contrast only 2 out of 20 with faecal pH 7.5 developed *E. histolytica* infection. Significantly, the caecal contents of rats positive for *E. histolytica* infection possessed pH 7.5. The whitish adhesions on the caecal wall containing a very large number of trophozoites of *E. histolytica* possessed pH 6.5.

An attempt was made to identify the factors which could be responsible for the failure of *E. histolytica* to infect rats possessing caecal content of alkaline pH. Studies were carried out to determine the *in vitro* amoebicidal action of caecal contents of rats. The contents from each rat failing to develop the amoebic infection or normal ones were collected, suspended in 2.5 ml distilled water and their pH recorded. These were then added promptly to young actively multiplying culture of *E. histolytica* (B-1) growing in 2.5 ml monophasic culture medium (Inactivated bovine serum was diluted 1:7 with M/40 phosphate buffer containing 0.85% NaCl and particulate rice starch). Tests were carried out at pH 6.5 and also at pH 7.5 adjusted with N/10 NaOH.

Table 1 *Caecal amoebiasis of rats in relation to faecal pH.*

Range of faecal Ph	No. of rats inoculated	No. of rats with ulcers		Av. caecal score
	infected	3-grade	4-grade	
5.5–6.5	46/46	18	21	6.3
7.5–7.8	20/2	Nil	Nil	0