TABLE II

Determination of units of complement

Reaction Mixture	Dilution of Guinea-pig Complement							
	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
C′ + Ag + SSRBC	+	+	+	+	+	⊕	–	–
C′ + Ab + SSRBC	+	+	+	+	+	+	⊕	–
C′ + Ag + Ab + SSRBC	+	+	⊕	–	–	–	–	–
C′ + diluent + SSRBC	+	+	+	+	+	+	⊕	–
Units of Complement	64	32	16	8	4	2	1	½

⊕ indicated positive for 50% hemolysis.

the end of the incubation period hemolytic activity giving rise to at least 50% lysis of SSRBC was determined.

Quantitation

We define 1 unit of complement as the least amount of complement required to lyse 50% of 1% SRBC sensitized to 1 agglutinating unit. As shown in Table II, this unit is determined from the fourth series of tubes which have C′ as control by finding 50% hemolytic activity. Similarly, we can determine the end points of the other three series of tubes as illustrated in Table II.

The specific complement fixation due to antigen antibody reaction $= C'_{tot} - C'_{Ag} - C'_{Ab}$ where, C'_{tot}, C'_{Ag} and C'_{Ab} are units of complement consumed in the presence of antigen plus antibody, antigen alone, and antibody alone, respectively. As per Table II, for example, specific complement consumption due to antigen-antibody interaction $= (16–1) - (2–) - (1–1) = 14$. For the sake of convenience serial dilutions of complement have been considered. Instead, increasing dilutions of complement may be used to determine intermediate units of complement.

Discussion

The definition of one unit of complement helps in quantitating the amount of complement consumed by specific antigen antibody interaction. This aids in several assays where the antigen is anticomplementary, particularly in the case of tumor antigens. In defining the unit of complement, we have taken in account one agglutinating unit of SRBC which also quantities of amount of antibody coating the indicator SRBC. This ensures reproducibility of the amount of antibody on SRBC for all tests and hence assures uniformity in indicator cell sensitivity and standardization of Sheep Red Blood Cell lysis.

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POST HARVEST INDUCED CHANGES OF DIOSGENIN IN COSTUS SPECIOSUS SIMS

COSTUS speciosus Sims (Family—Zingiberaceae) is considered to be the potent source of diosgenin[1,2]. Sarin[3] *et al.* found that diosgenin content of this plant is lowest in the dormant rhizomes (0·63%) and highest at early flowering stage (2·61%). The post-harvest increase of 5 to 15% in the steroidal sapogenin from fresh tuber of Dioscorea belizensis on its incubation with water under defined condition have been reported by Blunden and Hardman[4]. Incubation of the harvested tuber of Dioscorea deltoidea and whole seeds of Trogonella foenumgraecum, wih indole 3 acetic acid and gibberelic acid increased the sapogenin yield upto 35%[5].

For systematic study of the effect of incubation on Costus rhizomes we grew, in our garden, plants from rhizomes collected from Khandala hills in Maharashtra. Rhizomes harvested from plants 60 days old 22" tall on 11–8–1977, giving very high increase in diosgenin content on incubation, is reported in this short communication.

Thin slices of freshly collected rhizomes were incubated with water and dilute solutions of growth regulator for 24 hours at 37° C and then analysed by the colorimetric method of Akira Akahori[6] and modified by Patel[7]. Results of this experiment are given in Table I. The table shows that the diosgenin content

TABLE I

Diosgenin content of Costus speciosus rhizomes on moisture free basis

Procedure	Per cent diosgenin	Per cent increase over control
1. Fresh tuber without incubation (control)	1·366	
2. Incubation with water	3·503	156·44
3. Incubation with 2 ppm IAA	3·234	136·74
4. Incubation with 20 ppm IAA	3·725	172·69
5. Incubation with 200 ppm IAA	4·460	226·50
6. Incubation with 1 ppm GA	3·234	136·74
7. Incubation with 10 ppm GA	3·195	133·89
8. Incubation with 100 ppm GA	3·946	188·87
9. Incubation with 1 ppm 2 4-D	4·081	198·75
10. Incubation with 10 ppm 2, 4–D	4·235	210·02
11. Incubation with 100 ppm 2, 4–D	5·050	269·69

IAA = Indole 3-acetic acid, GA = Gibberellic acid, 2, 4–D = 2, 4 dichloro phenoxy acetic acid.

1·3% in the control increases to 3·5% with water incubation and to 5·0% on incubation with 2, 4–D. The Maximum increase in yield is 270% over the control.

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ANTHRACENE DERIVATIVES IN LEAVES AND FRUITS OF *CASSIA ALATA*

Cassia alata (Family—Ceasalpinaceae) is a native of tropical America but is now widely distributed in the tropics *e.g.* in Western and Eastern Africa[1]. It is a soft wooded shrub up to 15 feet high. The fruits of this plant consist of a pod of up to 6 inches long containing 30–60 seeds.

Reduced anthraquinone compounds are reported to be present in leaves[2] and quinone pigments[3] in roots of *Cassia alata*. Laxative action of leaves has been confirmed by animal experiments[4]. The perusal of literature has revealed that there is no report on active constituents of fruits of this plant. In view of cathartic values of anthraquinones, both leaves and fruits of *Cassia alata* were systematically examined for the presence of anthraquinone compounds.

Experimental

Anthracene derivatives isolated from leaves and fruits of *Cassia alata* are listed in Table I.

TABLE I

Nature and concentration of anthraquinones compounds isolated from leaves and fruits of Cassia alata

	Free oxidised aglycones	% w/w dry wt.	Aglycones (present as glycosides)	% w/w dry wt.	Total % w/w dry wt.
Leaves	Aloe emodin	0·1	Rhein Aloe emodin	0·1	0·2
Fruits	Rhein Aloe emodin Emodin	0·3	Rhein Aloe emodin Emodin	1·0	1·3

Procedures for extraction of free and combined anthraquinones from leaves and fruits of *Cassia alata* were similar as *Cassia siamea*[5]. Extracts were examined by thin layer chromatographic processes and separated components were isolated by preparative thin layer chromatography[0]. A colorimetric method[6] was used to esti-