ar d tri-*o*-methylgallaldehyde[8]. Selenium dioxide oxidation[9,10] of the chalkone (V) yielded 5, 6, 7, 8, 3′, 4′, 5′-heptamethoxyflavone (I). The synthetic flavone (I) was identical in properties with those recorded for the flavone isolated[1] from *Eupatorium coelestinum* thereby confirming its proposed constitution.

Experimental

2-(3′, 4′, 5′-Trimethoxy) benzoyloxy-3, 4, 5, 6-tetramethoxyacetophenone (III) : A mixture of 2-hydroxy-3, 4, 5, 6-tetramethoxyacetophenone (II) (2 g), tri-*o*-methylgalloyl chloride (2·5 g) and dry pyridine (15 ml) was heated at 100° for 1 hr. The ester (III) was extracted with ether and purified. On removal of the solvent, III was obtained as a liquid (1·8 g) which did not solidify and hence used as such (Found : C, 58·3; H, 5·5. $C_{22}H_{26}O_{10}$ requires C, 58·66, H, 5·82%).

NMR (δ, *CDCl₃, TMS as internal standard*) : 2·48 (3H, s, $-COCH_3$), 3·84 (3H, s, $-OCH_3$), 3·94 (12H, s, 4X $-OCH_3$), 3·98 (6H, s, 2X $-OCH_3$), 7·44 (2H, s, $C_{2'}-H$ and $C_{6'}-H$).

2-Hydroxy-3, 4, 5, 6, 3′, 4′, 5′-heptamethoxydibenzoylmethane (IV) : The above ester (III) (1·5 g), powdered potassium hydroxide (2 g) and pyridine (20 ml) were thoroughly shaken at 80° for 1 hr. The β-diketone (IV) (1·3 g) that was worked up as usual, but it did not solidify and was thus used as such after purification (Found : C, 58·5; H, 5·7. $C_{22}H_{26}O_{10}$ requires C, 58·66, H, 5·82%). It dissolved in aqueous sodium hydroxide (10%) and gave an olive-green colouration with alcoholic ferric chloride.

5, 6, 7, 8, 3′, 4′, 5′-Heptamethoxyflavone (I) : The above β-diketone (IV) (1 g); fused sodium acetate (1·2 g) and glacial acetic acid (5 ml) were refluxed for 3 hr. The flavone (I) thus obtained, crystallised from chloroform-petroleum ether as colourless needles (0·8 g), m.p. 104–5° (Found : C, 60·9; H, 5·9. $C_{22}H_{24}O_9$ requires C, 61·10; H, 5·59%).

IR (*KBr, cm⁻¹*) : 1630 (conj. ketone), 1580, 1500 (aromatic).

NMR (δ, *CDCl₃, TMS as internal standard*) : 3·92 (3H, s, $-OCH_3$), 3·95 (12H, s, 4X $-OCH_3$), 4·01 (3H, s, $-OCH_3$), 4·08 (3H, s, $-OCH_3$), 6·60 (1H, s, C_3-H). 7·12 (2H, s, $C_{2'}-H$ and $C_{6'}-H$).

2′-Hydroxy-3, 4, 5, 3′, 4′, 5′, 6′-heptamethoxychalkone (V) : A solution of 2-hydroxy-3, 4, 5, 6-tetramethoxyacetophenone (II) (1 g) and 3, 4, 5-trimethoxybenzaldehyde (1·5 g) in ethanol (10 ml) was treated with aqueous-ethanolic potassium hydroxide (1·2 g) at room temperature for 48 hr. The chalkone (V) thus obtained did not solidify and was used as such after purification (1·1 g) (Found : C, 60·6; H, 5·9. $C_{22}H_{26}O_9$ requires C, 60·82; H, 6·03%). It gave brown ferric reaction.

NMR (δ, *CDCl₃, TMS as internal standard*) : 3·80 (3H, s, $-OCH_3$), 3·87 (9H, s, 3X $-OCH_3$), 3·90 (9H, s, 3X $-OCH_3$), 6·76 (2H, s, C_3-H and C_8-H), 7·76 (2H, s, $C\alpha-H$ and $C\beta-H$).

5, 6, 7, 8, 3′, 4′, 5′-Heptamethoxyflavone (I) : A mixture of the above chalkone (V) (0·8 g), selenium dioxide (0·5 g) and iso-amyl alcohol (20 ml) was refluxed for 72 hr and the reaction product was worked out as usual. The flavone (I) thus obtained, crystallised from chloroform-petroleum ether as colourless needles (0·5 g), m.p. 104–5°. It agreed with the synthetic sample obtained by the earlier method.

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PASSIVE BACTERIAL AGGLUTINATION FOR THE DETECTION OF HEPATITIS B VIRUS SURFACE ANTIGEN

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The detection of hepatitis B surface antigen (HBsAg) has become very important in clinical practice, blood bank service, and epidemiological studies[1,2]. Most laboratories in India use counterimmunoelectrophoresis (CIE) for this purpose[3,4]. This technique is simple and highly specific but only moderately sensitive. There are three methods with high sensitivity available currently, namely radioimmunoassay, enzyme linked immunosorbent assay and reversed passive haemagglutination. Although research laboratories are importing reagents and using these methods, they are highly expensive and exacting to be recommended for routine use in hospital-based clinical laboratories[5,6]. Therefore we have developed a simple, rapid, specific and

TABLE I

HBsAg titres of two sera tested by CIE and PBA

Number	The titre* of HBsAg in sera by	
	CIE	PBA
1.	16	512
2.	128	4096

* The reciprocal of the highest dilution found positive.

TABLE II

Comparison of 100 *sera tested for HBsAg by CIE and PPA*

	CIE positive	CIE negative	Total
PBA *positive*	1	6	7
PBA negative	0	93	93
Total	1	99	100

highly sensitive method using Cowan I strain of *Staphylococcus aureus* for passive agglutination. This organism is rich in protein A which is a receptor for the Fc portion of immunoglobulin molecules. After the bacteria are coated with immunoglobulins of the desired specificity, they form a convenient reagent for the detection of antigen as it will agglutinate the bacteria[7].

The bacteria were grown in Todd-Hewitt broth at 37° C for 18 hours, harvested, washed in phosphate buffered saline (PBS, pH 7·4, 0·03M) and suspended in PBS (10% v/v). It was treated with formaldehyde (0·3% final concentration) washed again and resuspended as above and heated at 56° C for 30 minutes[8]. The cells were again washed and resuspended in PBS containing 0·1% sodium azide, and stored at 4° C as stabilized bacteria. Human antibody to HBsAg was collected from suitable donors and used to coat the bacteria[8]. Coating was done by mixing 1 volume of antiserum with 10 volumes of the 10% stabilized bacterial suspension and incubating at 30–33° C for 30 minutes. The coated cells were resuspended (10% v/v) in PBS with 0·1% sodium azide and stored at 4° C.

Sera to be tested for the presence of HBsAg were first absorbed with stabilised bacteria to remove any antistaphylococcal antibody. Twenty microlitres of each of the absorbed sera and equal volumes of 1% suspension of bacteria coated with anti-HBs were placed within wax circles of 2 cm diameter, on clean glass slides. After 2 minutes of continuous shaking, the mixtures were examined under appropriate lighting conditions for visible bacterial agglutination. This method is termed passive bacterial agglutination (PBA).

Two sera positive for HBsAg by CIE were serially diluted and the dilutions were tested by both CIE and PBA. The results are presented in Table I. The new method is about 30 times more sensitive than CIE.

Sera from 100 blood donors were also tested simultaneously by both methods, and the results are presented in Table II. While only one serum was positive by CIE, 7 were found to be positive by PBA.

This method would be suitable for routine diagnostic testing of human sera and other body fluids for the presence of HBsAg. It is simple, rapid, specific and highly sensitive.

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BONNET MONKEY ERYTHROCYTE ROSETTING: A MARKER FOR 'B' LYMPHOCYTES

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ROSETTING techniques have been widely employed for identifying cell surface markers of human lymphocytes[1–3]. Lymphocytes modulated by thymic influence (T-cells) rosette with sheep erythrocytes (SE) and thymus-independent lymphocytes (B cells) rosette with SE coated with antibody and complement (SEAC)