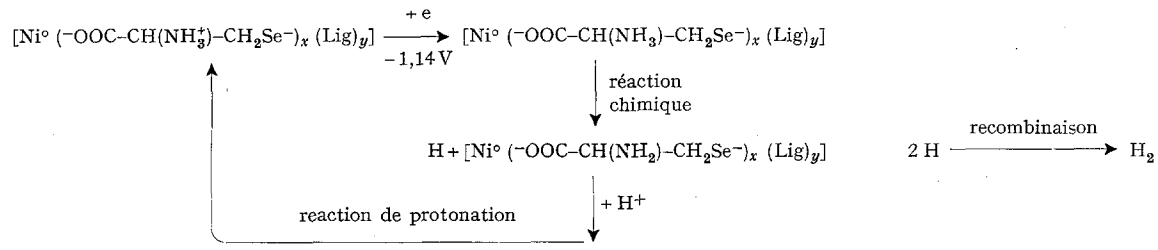
la charge de l'atome central dans le complexe catalytique nous expliquons le mécanisme de la décharge catalytique de l'hydrogène par la réaction suivante:



Dans ce schéma x représente le nombre des molécules de séléno-cystéine qui entre dans la composition du complexe. Les valeurs les plus probables de x sont 1 ou 2[1]. Nous avons noté par Lig les molécules d'eau ou de composant de tampon (ion acétate) qui peuvent entrer dans la composition du complexe catalytique. Compte tenu du nombre de coordination du nickel, $x+y = 6$ et donc les valeurs les plus probables de y sont 2 ou 4.

Summary. It has been found that seleno-cystine, reduced to seleno-cysteine and bound in ligand form to nickel ion, produces catalytic hydrogen discharge in slight acid media. This discharge occurs in the region of a catalytic prewave located at more positive potentials ($E_{1/2} = -1.14$ V, S.C.E.) compared with the normal wave of H_3O^+ ($E_{1/2} = -1{,}67$ V, S.C.E.).

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Anthraquinone Glycoside from the Seeds of *Cassia occidentalis* Linn.

Cassia occidentalis[1], commonly known as Kasondi in Hindi, is one of the 40 species of the genus *Cassia* (N.O. Leguminosae). It is a diffuse undershrub and distributed throughout India. The plants of this genus have been widely used for their acid, mucilagenous and cathartic properties. The seeds are bitter and used for winter cough and as a cure for convulsions in children. The roasted seeds are an excellent diuretic. The plant enters into the composition of numerous ointments prescribed in skin diseases. A number of workers have chemically investigated the seeds[2–5] and roots[6] of the plant but none have reported the presence of glycoside in this plant. Rizvi et al.[7] have, however, reported the presence of sterol glycoside in seeds. Isolation and identification of anthraquinone glycoside from the seeds is reported here.

The powdered seeds of *C. occidentalis* (8 kg) were successively and exhaustively extracted with petroleum ether (60–80°), chloroform and ethanol in soxhlet extractor. Ethanolic extract was concentrated and further fractionated with petroleum ether, benzene and ethyl acetate. Ethyl acetate extract was concentrated and recrystallized from methanol into orange crystals m.p. 230–32°, yield 700 mg. Analysis found: C 58.68; H 5.21. calcd. for $C_{22}H_{22}O_{10}$ C 59.19; H 4.93. The purity of the compound was tested on paper with butanol: acetic acid: water (4:1:5) whereupon a single spot was obtained. It gave all the colour reactions[8,9] of anthraquinones and also gave Molich's test, thereby showing its glycosidic nature. Acetyl derivative of the compound was prepared and recrystallized from methanol as light yellow needles m.p. 141–43°. The compound was hydrolyzed with 10% methanolic sulphuric acid to give an aglycone and sugar. The sugar was identified to be glucose by paper chromatography and by preparing its osazone derivative.

The aglycone was recrystallized from petroleum ether: benzene (1:1) mixture as golden yellow needles, m.p. 204–06°, analysis, found, C 67.14; H 4.5. Calcd. for $C_{16}H_{12}O_5$ C 67.6; H 4.2. Mol wt. by Rast method is 278, calculated for $C_{16}H_{12}O_5$, 284. It was soluble in benzene, chloroform, ethyl acetate and petroleum ether. Acetyl derivative of the aglycone was prepared and recrystallized from methanol into yellow green needles, m.p. 185–87°. Acetyl group estimation showed the presence of 2 hydroxyl groups in the compound. The aglycone contains 1 methoxyl group as determind by semi-micro method of Belcher et al.[10]. The compound was insoluble in 5% aqueous sodium carbonate solution, thereby showing the absence of any free β-hydroxyl group in it. λ_{max} at 435 nm also indicated the presence of two α-hydroxyl groups[11,12]. In the IR-spectra, 2 distinct peaks at 1680 cm^{-1} and 1620 cm^{-1} have been observed which are characteristic[13] of non-chelated and chelated carbonyl groups respectively. Therefore, both hydroxyl groups are either at 1:8 or at 4:5 position. On demethylation, emodin m.p. 254–55° was obtained. From all these studies, the aglycone was identified to be physcion which was further confirmed by the m.m.p. and superimposable IR-spectra with an authentic sample of physcion.

The original compound is, therefore, proved to be a glucoside of physcion. Periodate oxidation of the glucoside

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followed by the estimation of formic acid liberated and uptake of periodate showed the presence of only one glucose moiety in the glucoside.

Methylation of glucoside followed by the acid hydrolysis resulted in the isolation of a compound m.p. 176–78°, it was recrystallized from methanol as fiery red meedles, m.p. 177–78°. The compound was identified to be β-methyl physcion[14], m.p. 178°.

Isolation of β-methyl physcion from the hydrolysis of methylated glucoside indicates that free hydroxyl group is at position 8 in the glucoside. This indicates that glucose moiety is attached at position 1. Hence glucoside has been identified as 1-glucoside of 1:8 dihydroxy-6-methoxy -3-methyl anthraquinone or 1-glucoside of physcion.

Zusammenfassung. In *Cassia occidentalis* Linn. wurde das 1-Glukosid des 3-Methyl-6-methoxy-1,8-dihydroxy-anthrachinons nachgewiesen.

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Structural Characterization of Immunoglobulins Contained in Polyacrylamide Gels

Polyacrylamide gel electrophoresis is widely used in biochemistry due to its good resolution. However, doubts often exist about the precise identitity of separated proteins. For some, specific enzymatic reactions[1,2] or satining methods[1–3] are available. Associations of polyacrylamide gel electrophoresis with immunodiffusion using specific antisera have been frequently used to identify proteins separated from a mixture[1,3–10].

As an alternative for the identification of immunoglobulins, a method will be described where the protein, contained in polyacrylamide gel slices, is reduced in the presence of sodium dodecyl sulphate (SDS), followed by electrophoretic determination of the molecular weight of the sub-units released to the supernatant.

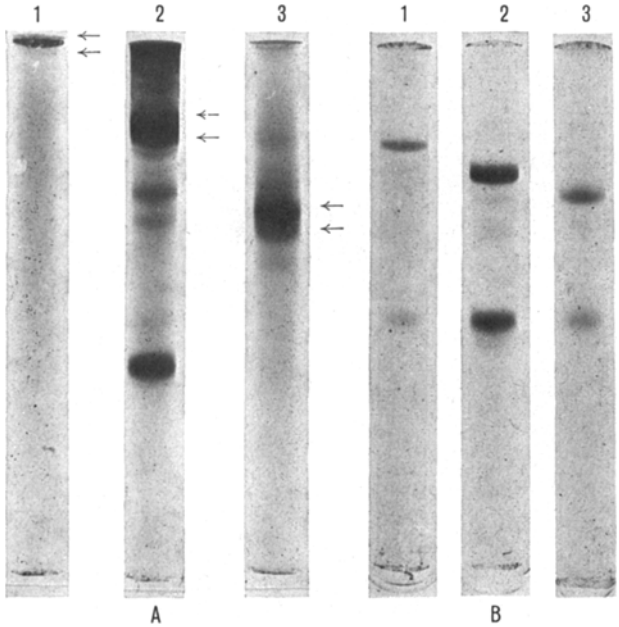

Fig. 1. Structural characterization of immunoglobulins contained in polyacrylamide gels. The 3-gel set A illustrates sodium dodecyl sulphate-polyacrylamide gel separation of 25 μg of a polymeric monoclonal IgM protein (gel 1), 75 μg of a partially purified dimeric monoclonal IgA protein (gel 2), and 50 μg of isolated polyclonal IgG (gel 3). The arrows indicate the regions sliced in twin, frozen gels, for reduction and alkylation. 2 to 3 slices of each gel were transferred to small test tubes, and 50 to 100 μl of sodium dodecyl sulphate, dithioerythritol – contaning buffer added to each tube. After reduction was completed, a supernatant was collected and run in the 3-gel set B. Gel 1 of this set correspond to reduction of IgM, gel 2 to reduction of IgA, and gel 3 to reduction of IgG. (For details, see text.)

Material and methods. Proteins. Immunoglobulins G, A, and M were isolated as described previously[11,12]. Chymotrypsinogen A and ovalbumin were purchased from Serva, human albumin from Fluka, and lactate dehydrogenase and catalase from Sigma.

Acrylamide electrophoresis. Electrophoretic separations were realized in 70×6 mm polyacrylamide gel discs, containing 0.1% (w/v) sodium dodecyl sulphate, according to SUMMERS, MAIZEL and DARNELL[13]. The initial separation of a given immunoglobulin was made on a 4.25% (monomer w/v) gel, and mol. wt. calculation was based on data collected from runs on 7.5% (monomer w/v) gel.

Reduction of proteins contained in acrylamide gel. Samples were run in duplicate, one gel cylinder to be frozen on dry ice, the other to be stained[10]. The position of the protein to reduce on the frozen gel was calculated from its mobility on the stained gel, after convenient correction for shrinking in the stain solution and individual differences among gels separated in the same run. The frozen gel was transversally sliced in 1 mm width sections, and 2 to 3 slices calculated to contain a given immunoglobulin were placed in a small test tube, to which 50 to 100 μl of 2% (w/v) sodium dodecyl sulphate in sodium phosphate buffer pH 7.2, 0.01 *M*, and 5 to 10 μl of 1 *M* dithioerythritol were added. Reduction was carried out at 100 °C for 2 periods of 10 min, separated by a 20 min period of reduction at room temperature. Proteins to be used as references were treated identically, except that

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