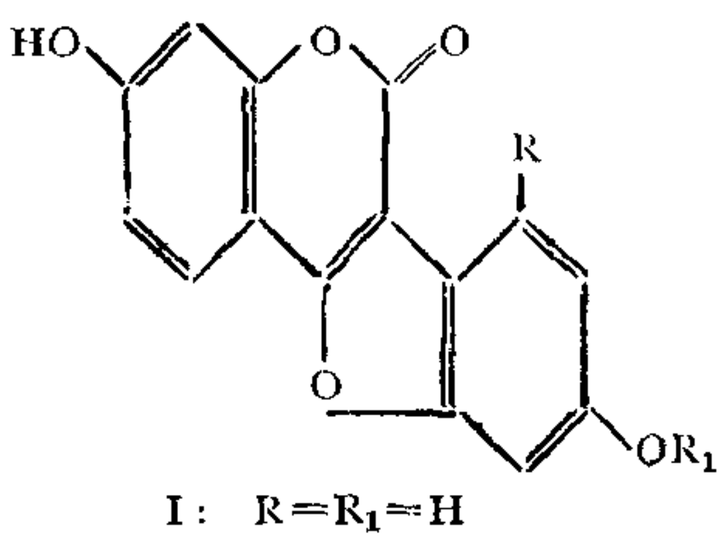
LETTERS TO THE EDITOR

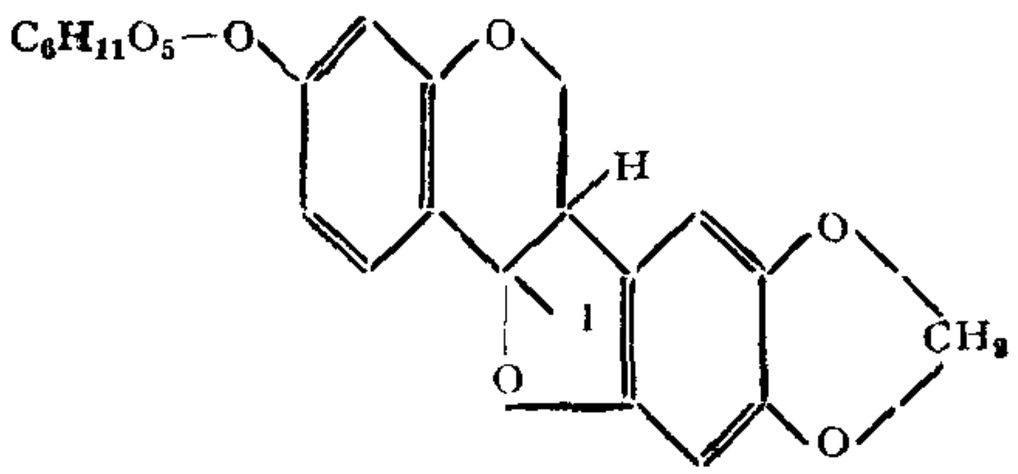
XANTHOSINE FROM *TRIFOLIUM ALEXANDRINUM* SEEDS

PAST work on several species of *Trifolium* has revealed the presence of different types of compounds, the most recent among which are coumestrol[1] (I), trifoliol[2] (II), and trifolirhizin[3] (III). In connection with our studies on 3-phenylcoumarins we have now undertaken a chemical investigation of *Trifolium alexandrinum*. It is also known as Egyptian clover and is not native to India. The plant is grown during the winter months. For the present work the seeds were obtained from the Indian Agricultural Research Institute, New Delhi.



I: $R=R_1=H$

II: $R=OH$; $R_1=CH_3$



III

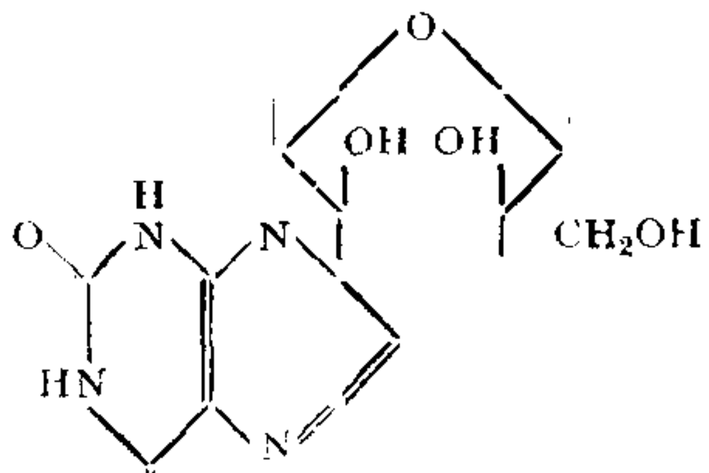
***Petroleum Ether Extract*: *β*-Sitosterol.**—Finely ground sample was extracted in succession, in a soxhlet, with petroleum ether, ether and alcohol. A final aqueous extract was also made. The oil obtained from the petroleum extract yielded, after chromatography over neutral alumina, *β*-sitosterol, m.p. 136–38°. This compound has been isolated earlier[4] from the same material.

Ether extract yielded a mixture which could be resolved into methanol-soluble and methanol-insoluble fractions. The latter, m.p. 262–65° (**Found**: C, 70·7; H, 10·7%) was a crystalline optically active ($[\alpha]_D^{19} - 54·3°$ in pyridine) compound answering the Lieberman-Burchard test. The ultra-violet spectrum in ethanol had maxima at 246, 250 and 262 mμ. The quantity was, however, too small for detailed studies. The methanol-soluble fraction appeared to be a mixture of flavonoids or related compounds but its resolution was difficult. It is being examined further.

Alcoholic Extract: *Xanthosine*.—The alcoholic extract on concentration deposited a colourless nitrogenous compound which could be readily crystallised from hot water. It did not melt up to 340° C. and exhibited a large negative rotation in pyridine $[\alpha]_D^{19} - 106°$. It had the molecular formula $C_{10}H_{12}O_6N_4$, 1 H_2O. Tests for alkaloids, amino-acids or peptides were negative. It answered the Molisch test but did not reduce Fehling's solution. On hydrolysis with 6 N hydrochloric acid it yielded glycine as the only recognisable product. Its ultra-violet spectrum in 0·1 N alkali $\{\lambda_{max.}$ [log ε] 248 (4·06) and 277 (4·01) mμ$\}$ was significantly different from that in 0·1 N hydrochloric acid $\{\lambda_{max.}$ [log ε] 235 (3·93) and 261 (3·97) mμ$\}$. The absorption curves resembled those of typical purines and were particularly similar to those of 9-methylxanthine and xanthosine.[5] The infra-red spectrum was also characteristic of purine derivatives.[6]

Boiling with 7% sulphuric acid yielded D-ribose which was identified by paper chromatographic comparison with an authentic sample. The free purine base (m.p. > 340°) could be obtained in a pure state by milder hydrolysis using 1 N acid and boiling for an hour. It has the following spectral characteristics: $\lambda_{max.}^{0·1\ N\ NaOH}$ 282 mμ and $\lambda_{max.}^{0·1\ N\ HCl}$ 263 mμ. Circular paper chromatographic examination with butanol-water (86 : 14) showed the presence of one component (R_f : 0·18). These properties corresponded to those of xanthine. The identity was confirmed by the preparation of the perchlorate, m.p. 262–63°. These results showed that the compound isolated from *T. alexandrinum* seeds is xanthosine (IV). The identification has been confirmed by comparison with a sample of xanthosine obtained from guanosine by nitrous acid treatment.[7] While the occurrence of xanthine in plants is fairly widespread, the present study seems to be the first report of isolation of free xanthosine from a plant source. Two other purine derivatives have been reported earlier in some other species

of *Trifolium*, guanosine from *T. pratense*[8] and uric acid from the seeds of *T. officinalis*.[9]



IV

Aqueous Extract : Galactomannan.—Treatment of the aqueous extract with Fehling's solution yielded an insoluble copper complex. The complex was decomposed by treatment with dilute hydrochloric acid. Subsequent dilution with ethanol yielded a colourless polysaccharide which could be purified by dissolution in water and reprecipitation with ethanol. It exhibited positive rotation ($[\alpha]_D^{19} + 72°$) in water. On hydrolysis it yielded galactose and mannose thus showing that it was a galactomannan.

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PREPARATION AND ARSORPTION SPECTRUM STUDIES OF RARE EARTH PONGAMOL COMPLEXES

RARE EARTH chelates have recently received considerable attention because of their possible application in the development of Lasers.[1] Much work has been done on the rare earth complexes of diketones, their preparation,[2] their spectral properties[3,4] and on their fluorescence spectra.[5,6] In a previous communication[7] from this laboratory the preparation and spectral properties of Europium pongamol complex were reported. In the present communication the preparation and spectral data of Samarium, Dysprosium, and Ytterbium chelates with pongamol are reported.

Preparation.—A weighed amount of hte rare earth oxide (99·9%) was dissolved in A.R. grade hydrochloric acid and the solution was evaporated on a steam bath to dryness. The dry mass was extracted with absolute ethanol (20 ml.) and a calculated amount of pongamol in absolute ethanol (30 ml.), mole ratio 1 : 3, was added to the rare earth chloride solution. The pH of the solution was adjusted by the addition of alcoholic ammonia just short of precipitation of the rare earth hydroxide. The precipitate obtained was filtered, washed with a little alcohol, dissolved in benzene and the solution was evaporated at room temperature. The compound obtained was vacuum-dried. The elemental analyses of the chelates indicate that the metal to ligand ratio is 1 : 3.

Spectral Data.—The absorption spectra of the ligand and the chelates in the ultraviolet region were recorded using Hilger-Watts spectrophotometer and in the infrared region using a Perkin Elmer Model 137 infracord. The compounds were examined as solids in Nujol mulls.

In the ultraviolet region the ligand in chloroform exhibits two absorption band maxima one at 350 mμ and the other at 250 mμ. In the present investigation the study of the absorption spectra of the chelates is confined to solutions in chloroform as the chelates are insoluble in methanol and ethanol. In the chelates investigated two band maxima could be located at 350 and 250 mμ, while a large intensification could be noticed in the relative ϵ values, in the case of Samarium and Dysprosium chelates. The $\lambda_{max.}$ and $\epsilon_{max.}$ values are detailed in Table I.

In the visible region the ligand has no characteristic absorption. The characteristic band maxima of Samarium ion at 402 mμ could not be located. However the characteristic band maxima of Dysprosium ion at 910 mμ (ϵ value 3·9) and that of Ytterbium ion at 980 mμ (ϵ value 5·1) could be located.

In the infrared region the diketone now investigated showed two bands at 1600 cm.$^{-1}$ and 1550 cm.$^{-1}$ These may be due to the enol-chelate and the perturbed carbonyl respectively. In the infrared spectra of metal diketonates