

- (I) ; R = COOH; R' = OH
 (II) , R = COOH, R' = OAc
 (III) ; R = COOMe, R' = OH

the pimarane type skeleton^{3,4}. (I) formed a monoacetate (II), m.p. 102–4°, C₂₂H₃₄O₄ (Found; C, 73.00; H, 10.02; C₂₂H₃₄O₄ required; C, 72.92; H, 9.39%, M⁺ at m/z 362); $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 1725; ¹H NMR (δ , TMS) 4.90 (CH–O–COCH₃) indicating the secondary nature of the hydroxyl group. The equatorial orientation of the sec. OH group was inferred by coupling shape of the carbinyl protons signal. (I) on treatment with CH₂N₂ yielded (III), m.p. 110° (dec.), C₂₁H₃₄O₃ (Found; C, 75.38; H, 10.00; C₂₁H₃₄O₃ required; C, 75.44; H, 10.18%; M⁺ 344); $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ 1710 ester group; ¹H NMR (δ , TMS), 3.00 (3H, 1 × COOCH₃) which was unaffected by boiling with grignard reagent in PhMe. This is a clear indication that carboxyl group is highly hindered position as in case of pimaric acid and its methyl ether^{3,5}. The carboxylic group at C-4 was supported by the comparative studies of the pimaric acid^{3,4}, with that of dipoloic acid. The dihydrodipoloic acid (IV) was obtained on hydrogenation of (I) with Pd–C which had similar ¹H NMR spectrum as in (I) except that signal of –C₂H₅ group at δ 0.92 (t, J = 9 Hz) in addition to the methyl signals indicating that vinylic group in (I) has reduced in (IV). Oxidation of (III) with MnO₂ in C₆H₆ afforded a ketone, m.p. 69–72°, (α)_D²⁸ –62° (CHCl₃), C₂₀H₃₀O₃ (Found; C, 75.50; H, 9.40; C₂₀H₃₀O₃ required; C, 75.46; H, 9.43%, M⁺ 318); UV $\lambda_{\max}^{\text{MeOH}}$ 249 nm; IR $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ principal peaks at 1722 and 1695. The ¹H NMR spectrum of this ketone exhibited a pair of ABX type quartets at δ 3.12 ppm (1H, J_{AX} = 19.00 Hz, J_{AB} = 19.00 Hz), 2.80 ppm (1H, J_{BX} = 5.5 Hz; J_{AB} = 19.00 Hz, H–C–CH₂–CO), a broad singlet at δ 6.65 ppm (1H, OC–CH) and a singlet at δ 2.75 (3H, 1 × COOCH₃). The physical data of this ketone is very much similar to the ketone obtained from pimaric acid as reported in the literature^{3,5}. Hence the dipoloic acid has the structure 7-hydroxy-pimara-(15:16) ene-19-oic acid (I).

All the m.p.s. are uncorrected.

Isolation and purification: The air dried and powdered stem of *D. pentagyna* (2 kg) was extracted with EtOH

under reflux for 180 hr. The total EtOH extract was concentrated to a small volume (150 ml) and poured into H₂O (500 ml). The water insoluble fraction was extracted with C₆H₆. The C₆H₆ extract deposited a brownish solid (2.2 g). This deposit was extracted with hexane. The hexane extract yielded (I) which was purified over Al₂O₃ column (C₆H₆: CHCl₃, 6:4 V/V) into a colourless solid mass (900 mg). Recrystallization with MeOH (BDH) afforded colourless needles (800 mg), m.p. 150–52° (dec.). (Found; C, 74.98; H, 10.01; C₂₀H₃₀O₃ required; C, 75.00; H, 10.00%).

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INDIRECT POTENTIOMETRIC DETERMINATION OF REDUCING SUGARS

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SEVERAL immobilized enzyme electrodes have been reported for the determination of carbohydrate^{1–3}. Recently, periodate selective electrode has been used to determine reducing sugars in blood serum⁴. However, these electrodes are difficult to fabricate and not commercially available. This paper describes the fabrication of a simple Cu-selective electrode and its application to determine reducing sugars in natural products, employing standard addition technique.

Fabrication of Graphite [Ag₂S–CuS] Electrode

A 2.5 cm long graphite rod taken from a D-type dry cell was inserted into a tightly fitting polythene tube by which 2 cm of the rod was protruding out. An 'araldite'

seal was applied to ensure that no seepage of solution occurred into the tube. The electrode was 'dip-coated' by keeping it into a saturated solution of sodium sulphide for an hour. It was then transferred to a saturated solution of silver nitrate and left for another hour. The electrode was washed several times with double-distilled water and ethanol until all silver ions are removed. The same procedure was repeated using saturated copper sulphate solution instead of silver nitrate to precipitate CuS on the same rod. A small amount of mercury was poured into the polythene tube and electrical contact was made to the pH meter by inserting a thin copper wire enclosed in plastic sleeves. The potentials were measured against a saturated calomel electrode.

Reagents

- (i) Complexing acetate buffer: The glacial acetic acid (11.8 ml) was dissolved in about 500 ml of water, 0.84 g of sodium fluoride was added and the pH of the solution was adjusted to 4.1 with sodium hydroxide (1 M) and then diluted to 1 litre.
- (ii) Stanley-Benedict (SB) solution was prepared as described by Kolthoff *et al*⁵. The reagent was 0.1 M in copper sulphate, 1 M in sodium carbonate and 0.2 M in citric acid.

Procedure

- (i) Determination of the slope of the graph: The slope factor (S) of the graphite [Ag₂S-CuS] electrode was determined from a calibration graph of E (mV) vs $-\log \text{Cu}^{+2}$, prepared by dilution of standard copper sulphate solution (0.1 M) by adding complexing buffer in a volume ratio of 1:1.
- (ii) Sample treatment with SB reagent: An aliquot of the standard (or unknown sample) glucose solution (1 mg/ml) solution was transferred into a 30 ml Kjeldhal flask, followed by 0.5 ml of SB reagent and diluted to 10 ml by adding water. After boiling for 10 min, the solution was cooled and filtered through a Whatman No. 42 into a 100 ml volumetric flask. The buffer (50 ml) was added and the filtrate was diluted to the mark with water.
- (iii) Standard addition technique: The filtrate (50 ml) was transferred into a 100 ml beaker and the potential was measured using graphite [Ag₂S-CuS] electrode and calomel as reference. Then 0.5

ml of copper sulphate solution (0.1 M) was added and the potential was measured again.

- (iv) Preparation of the calibration curve: The total concentration $[X]$ of unreduced Cu^{+2} for each glucose standard was determined from the observed potential change ΔE , following the equation for the standard addition technique.

$$[X] = \frac{\Delta C}{(\text{antilog } \Delta E/S - 1)}$$

where S is the slope factor and ΔC is the change in copper concentration after the addition of known solution. A calibration graph was prepared by plotting $[X]$ against mg of glucose.

- (v) Determination of reducing sugars in honey: About 1 g of honey was weighed accurately, dissolved in water and diluted to 100 ml.

Graphite [Ag₂S-CuS] electrode shows Nernstian response in the range of 10^{-1} to 10^{-5} M towards copper ions with a slope of 27 mV per 10-fold change in concentration. The limit of detection being 10^{-7} M. The response time is instantaneous for 10^{-1} to 10^{-3} M solutions and increases to a maximum of 20–30 sec for 10^{-4} – 10^{-5} M solutions.

Preliminary experiments showed (pH 4.1) that the graphite [Ag₂S-CuS] electrode exhibits nonlinear behaviour towards Cu^{+2} in the presence of sodium potassium tartrate (Fehling's solution) whereas in the presence of citric acid the electrode shows a Nernstian response to Cu^{+2} and the slope factor also remains the same (27 mV/decade of concentration). Hence, SB reagent is preferred over Fehling's solution. Besides, glucose and fructose give the same reduction value with SB reagent for a boiling time of 10 min.

The optimal boiling time and the volume of SB reagent was 10 min and 0.5 ml respectively. Shorter boiling time results in the formation of a colloidal suspension of Cu(I) oxide. The volume of SB reagent larger than 0.5 ml decreases the sensitivity of the standard additions technique.

Sodium acetate-acetic acid complexing buffer (pH 4.1) was chosen to determine unreduced Cu^{+2} . This buffer not only adjusts the pH and ionic strength but also serves to decomplex Cu from all but very strong complexing agents⁶.

The proportionality between concentration of unreduced Cu^{+2} and glucose is linear in 0.25–2.5 mg of glucose. The relation was nonlinear at lower and higher concentrations. Therefore, the reducing sugar concentration of the sample should be brought into this range.

Table 1 Determination of reducing sugars in honey.

Brand of Honey	Graphite [Ag ₂ S-CuS] electrode (%)	Lane and Eynone's method. (%)
A.	11.6	11.6
B.	25.0	25.0
C.	25.7	25.6

(average of four determinations)

The standard deviation of the proposed method was 0.0075 for 0.5 mg of glucose in the series of six determinations. The method was also applied to determine the different brands of honey in the market. Several samples were determined by both the proposed method and the Lane and Eynone's method (table 1). There is satisfactory agreement between the results obtained by these two methods.

The routine methods of analysis to determine sugars in a variety of samples are mostly based on the reducing action of sugars in alkaline copper sulphate, involving gravimetric or volumetric⁷⁻⁸ techniques which are generally time-consuming. In the present method an ion-selective electrode is utilized for the determination of reducing sugars. The unreduced Cu(II) is directly related to amount of reducing sugars under specific conditions, using a calibration graph prepared with standard glucose solutions.

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TERMITE MOUNDS IN GEOCHEMICAL PROSPECTING

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THERE is extensive literature on termites and their structures dealing with their biological^{1,2} and ecological³ aspects; but studies on their geological aspects are scanty and scattered. Varahamihira (A.D. 505-587), a renowned astronomer, in his magnum opus *Brihat Samhita*, discussed the hydrological significance of the termite mounds⁴. A few recent studies dealt with the microstructures developed during the diagenesis of the termite mounds⁵, and with their magnetic properties⁶. The present study deals with the possible use of the termite mounds (figure 4) as a guide to locate underground ore deposits. For this purpose, the termite mounds occurring in the mineralised zones of chromium, vanadium, and copper, were collected from the following areas:

(i) Chromite in Kondapalli, (figure 1) included in the Survey of India toposheet No. 65 D/10. Here, the geological formations consist of khondalite and charnockite suite of rocks and ultramafics; these rocks are traversed by quartz and pegmatite veins. Geology of the area has been studied by earlier workers^{7,8}.

Chromite occurs as massive bodies, irregular veins, and lenticular and pocket-like lenses. Chromite occurrences and their mineralogical characters have been studied by earlier workers⁸⁻¹⁰. An area of 16 km² is covered for collecting samples of soils and termite soils in the mineralised zone and its surrounding country.

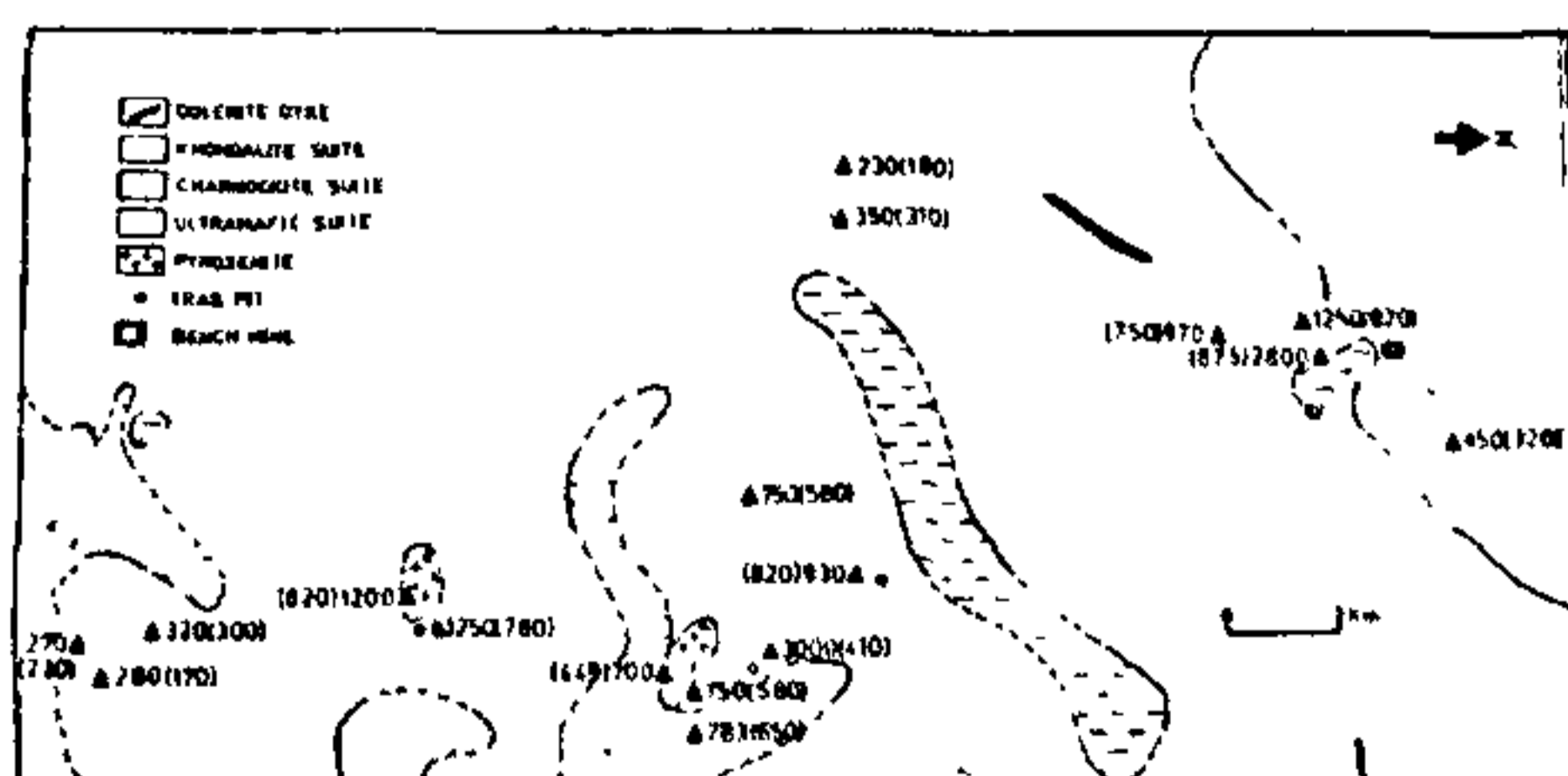


Figure 1. Geological map of the Kondapalli chromite mining area. In figures 1, 2 and 3, the concentrations of the ore elements are given for the termite mounds, the values given in parenthesis are those of their adjoining surface soils.