CHEMICAL INVESTIGATION OF COMMIPHORA MUKUL ENGL. (BURSERACEAE)[1]

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(Received May 28, 1966)

From an ethanolic extract of *C. mukul* Engl., two pure, crystalline compounds having m.ps, 83-84° and 137-38°, and molecular formulae $C_{30}H_{62}O$ and $C_{29}H_{50}O$ respectively have been isolated. Their acetyl and phenylurethane derivatives have been prepared. From a study of the chemical and physical characteristics of the compounds and their derivatives, the two compounds have been identified as myricyl alcohol and β-sitosterol. In addition to this, the ethanolic extract of the plant has been found to contain fifteen amino-acids which have been identified. Appreciable quantities of sucrose, glucose and fructose have also been found in the extract.

Introduction

Commiphora mukul Engl., locally known as *guggal* or *guggal-mukul*, is a small thorny shrub occurring commonly in the arid rocky tracts of Sind, Baluchistan, Rajasthan, Mysore and Madhya Pradesh. The ash-coloured bark of the plant comes off in rough flakes exposing the underbark which also peels off in thin papery rolls. On incision of the bark, an oleo-gum-resin is obtained as an exudate (about 1½-2 lb from each plant) which has been claimed to be of considerable medicinal value.[1] The resin has a bitter aromatic taste and balsamic odour. Of its most important medicinal properties are its uses as an intestinal disinfectant and its prescription for diseases such as the chronic catarrh of the bowels, chronic colitis, diarrhoea etc. As an antiseptic, it is used for mouth-wash and gargle, and in chronic tonsilitis, pharyngitis, ulcerated throat, etc.

Search of the literature so far has revealed that all the previous work[2] on this plant was related to the oleo-gum-resin; it was therefore thought useful to make a systematic chemical investigation of the plant. In doing this, the authors extracted the plant (excluding leaves and fruits) with alcohol and the extract, after removal of the solvent, was partitioned between water and ether. A small portion, mainly consisting of tannins which did not dissolve in either of the solvents, was not investigated. The water-soluble (43.8% of the total extract) and ether-soluble fractions (46.5% of the total extract) were investigated separately.

The water-soluble fraction was a clear brownish red syrup which was found to consist mainly of amino-acids and sugars. No alkaloid could be detected in it with the use of the usual reagents. Fifteen amino-acids were detected in the water-soluble syrup and they were identified by two dimensional paper chromatography (see Experimental). Among sugars, only sucrose, glucose and fructose were found to be present.

The ether-soluble fraction was a dark-green sticky mass which was saponified with alcoholic alkali. Chromatography of the unsaponifiable portion and subsequent fractional crystallisation led to the isolation of two pure crystalline compounds, m.ps, 83-4° and 137-38°. The former was found to possess the molecular formula, $C_{30}H_{62}O$ (Found: C, 82.02 and H, 14.28%; Calc. for $C_{30}H_{62}O$: C, 82.11 and H, 14.24%). It was optically inactive. The saturated nature of the compound was revealed by the fact that it did not absorb bromine in chloroform solution or hydrogen in presence of PtO_2 catalyst. Its infrared spectrum in KBr showed an absorption at 3413 cm^{-1} indicating the presence of a hydroxyl group. With acetic anhydride and pyridine at room temperature, the compound gave a crystalline acetate, m.p. 69-70°; and with phenyl isocyanate, a phenylurethane derivative, m.p. 98-99°; (Found: N, 2.60%; Calc. for $C_{30}H_{61}O.CO.NH.C_6H_5$: N, 2.51%). Reference to the literature revealed that all the above findings are in agreement with those of myricyl alcohol (see Experimental). It is, therefore, concluded that the compound with m.p. 83-4° is myricyl alcohol.

The compound with m.p. 137-38° was found to be a sterol because it gave a positive test with the Liebermann-Burchard reagent and also formed a digitonide, m.p. 246°. Its molecular formula was $C_{29}H_{50}O$ (Found: C, 83.75 and H, 11.92%; Calc. for $C_{29}H_{50}O$: C, 83.99 and H, 12.15%) and it had $[\alpha]_D^{31}$ −36.5°. The compound absorbed bromine in chloroformic solution indicating its unsaturated nature. Infrared spectrum in KBr showed a strong hydroxyl absorption at 3390 cm^{-1}. With acetic anhydride and pyridine, the compound formed an acetate, m.p. 134° $[\alpha]_D^{31}$ −28° and, with phenyl isocyanate, gave a phenylurethane derivative, m.p. 167-68°. The IR spectrum of the acetate did not show the presence of any hydroxyl group but two peaks at 1733 cm^{-1} and 1253 cm^{-1} appeared in the spectrum due to the acetyl group.

The above findings suggested the possibility of the compound being β-sitosterol and, in fact, the mixed melting points of the compound (m.p. 137-38°), its digitonide and the acetate with the authentic β-sitosterol and its corresponding authentic derivatives showed no depression. The infrared spectrum of the sterol was also found to be identical with that of authentic β-sitosterol.

Experimental

Combustion analyses were done by Dr. Alfred Burnhardt, 433 Mulheim, Ruhr, West Germany. Microhydrogenation was carried out by Dr. F. Pascher, Buschstrasse 54, Bonn, West Germany.

Extraction of the Plant

The plant (3 kg) without leaves, flowers and fruits, was cut into pieces and soaked in 95% ethanol (6 l.). The first extract was taken out after seven days and more alcohol added to the plant material. Three extracts were taken out similarly each after seven days. The combined extracts, on being freed from solvent, left a dark brownish-green sticky residue which weighed 120 g (4% of the weight of the plant). The total residue was treated with water (1 l.) and successively extracted with ether using 1.5 l. portions each time. A small fraction did not go in either of the solvents and was rejected. The ethereal extracts were washed with water and the washings combined with the main aqueous extract. It was then dried (anhyd. Na_2SO_4), filtered and freed from solvent. The residue was a dark green sticky mass which weighed 55.8 g (46.5% of the total extract). The aqueous fraction was warmed on the water bath in order to remove the dissolved ether, filtered and then completely freed from solvent under reduced pressure. The residue, thus obtained, weighed 52.5 g (43.8% of the total extract).

Water-soluble Fraction

It was a clear brownish-red syrup and its solution showed a pH of about 4.0. It gave a very small amount of precipitate with the potassium bismuth iodide reagent. When the aqueous solution was basified with ammonium hydroxide or sodium hydroxide, its colour darkened from light yellow to brownish-yellow and no precipitate was formed. With potassium iodide or picric acid, it did not give any precipitate.

Sugars

The aqueous solution was found to reduce Fehling's solution. Paper chromatography by the descending method, using n-butanol (20)/acetic acid (10)/water (10), and n-butanol (10)/pyridine (3)/water (3) as solvent mixtures and aniline oxalate and silver nitrate as spraying agents, showed three distinct spots which were due to sucrose, glucose and fructose. The identification was done by running chromatograms with authentic samples of these sugars. The spot of sucrose was cut out from a chromatogram and eluted with water. The eluate was hydrolysed with dilute hydrochloric acid and the hydrolysate, on paper chromatography, showed two spots of glucose and fructose while the spot due to sucrose completely disappeared.

Amino-acids

The aqueous fraction was chromatographed on paper by two-dimensional method, using n-butanol (100)/acetic acid (22)/water (50) as the solvent mixture and ninhydrin (0.2% in acetone) as the spraying agent. The following amino-acids were found to be present:

1. Cystine
2. Histidine
3. Lysine
4. Arginine
5. Aspartic acid
6. Serine
7. Glutamic acid
8. Threonine
9. Alanine
10. Proline (traces)
11. Tyrosine
12. Tryptophane
13. Valine (traces)
14. Leucine & Isoleucine (traces)

Ether-soluble Fraction

55.8 g of the ether-soluble fraction was saponified by refluxing with 6% alcoholic KOH (400 ml) for 3 hours. After this, the alcohol was completely removed and the residue treated with water (1.5 litre) and the unsaponifiable matter extracted thrice with ether. The combined ethereal extract was washed with water, dried and freed from solvent whereby a residue of the unsaponifiable matter (29.07 g. i.e., 52.1% of the ether-soluble fraction) was obtained.

The soap solution was treated with 25% H_2SO_4 when the free fatty acids precipitated out. The total fatty acids weighed 16.34 g (29.3% of ether-soluble fraction).

Isolation of Myricyl Alcohol and β-Sitosterol

The unsaponifiable matter was chromatographed on a column of neutral alumina, the elution being done successively with petroleum ether, benzene, chloroform and methanol. The first fraction of petroleum-ether eluate, on removal

of the solvent, gave a small amount of a colourless oil which was not investigated. The other petroleum-ether fractions yielded a pale yellow coloured essential oil having a faintly aromatic odour. Rechromatography of the later petroleum-ether fractions on a column of neutral alumina gave a small amount of a colourless, semi-solid mass which crystallised from acetone. Its m.p. was 83-84° and the yield was 25 mg. Combustion analysis of the compound gave values which were in agreement with the molecular formula, $C_{30}H_{62}O$ (Found: C, 82.02 and H, 14.28%; Calc for $C_{30}H_{62}O$: C, 82.11 and H, 14.24%). The compound was optically inactive and did not absorb bromine in chloroform solution. 1.529 mg of the compound in acetic acid solution absorbed 0.00222 mg of hydrogen in presence of PtO_2 catalyst at 25.5°C. IR spectrum of the compound in KBr showed an absorption peak at 3413 cm^{-1}. The compound was acetylated with acetic anhydride and pyridine at room temperature and the acetate crystallised from methanol. The m.p. of the acetate was 69-70° (lit. m.p. of the acetate of myricyl alcohol,[3] 69°C).

A solution of the compound in dry benzene was treated with phenyl isocyanate and the mixture left for two days. The solvent was then completely removed and the residue extracted with hot petroleum ether. The phenylurethane was crystallised from methanol-pet. ether and it melted at 98-99° (lit. m.p. of the phenylurethane of myricylalcohol,[4] 98°C). Found: N, 2.60%; $C_{30}H_{61}O.OC.NH.C_6H_5$ requires: N, 2.51%.

The benzene eluates from the alumina column were freed from solvents and the residues combined and recrystallised several times from methanol-chloroform until the melting point of the crystallisate was constant—small, colourless, shining needles, m.p. 137-38°, $[\alpha]_D^{31}$ -36.5° (c=1, $CHCl_3$), yield 1.8 g (Found: C, 83.75 and H, 11.92%; $C_{29}H_{50}O$ requires: C, 83.99 and H, 12.15%). The compound absorbed bromine in chloroform solution and the bromo-product was soluble in chloroform. With the Liebermann-Burchard reagent, it gave, at first, a violet colour which gradually turned into blue and then finally into a deep green colour. The infrared spectrum of the compound in KBr showed a strong peak at 3390 cm^{-1} and the spectrum was found to be identical with that of β-sitosterol. Also, an intimate mixture of the compound and authentic β-sitosterol in 1:1 proportion did not show any depression in melting point.

When a solution of the compound was treated with a solution of digitonin in ethanol, a digitonide was precipitated which melted at 246° (no depression with authentic β-sitosterol digitonide).

The acetate of the compound was prepared by reacting the compound with acetic anhydride in presence of pyridine. It was crystallised from benzene-ethanol in the form of transparent, shining leaflets, m.p.134°, $[\alpha]_D^{31}$-28° (c=1, $CHCl_3$). Found: C,81.03 and H, 11.23%; $C_{29}H_{49}OCO\,CH_3$ requires: C, 81.52 and H, 11.48%. Infrared spectrum of the acetate in KBr showed strong peaks at 1733 cm^{-1} and 1253 cm^{-1}. A mixed melting point of the acetate with an authentic sample of β-sitosterol acetate did not show any depression.

The phenylurethane derivative of the compound was prepared in the manner described above. The derivative was crystallised from methanol-pet. ether in clusters of small, shining needles, m.p. 167-68° (lit. m.p. of the phenylurethane derivative of β-sitosterol,[5] 166°C). Found: C, 81.01; H, 10.38 and N, 3.67%; $C_{29}H_{49}OCONH.C_6H_5$ requires: C, 81.00, H, 10.39 and N, 2.62%.

Acknowledgment.—The authors wish to thank Mr. M. Tasnif, Central Laboratories, PCSIR, for the supply of the plant material.

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