

Elutions from pet. ether-benzene (1:2) yielded compound III mp. 196°, $C_{32}H_{52}O_2$ M^+ (m/z) 468, which gave Liebermann-Burchardt test⁶ indicating triterpene nature. The IR spectrum (KBr) of III showed bands at 1730 cm^{-1} (acetate carbonyl), 1450 cm^{-1} (methylene group), 1380 and 1365 cm^{-1} (gem dimethyl group), 975 cm^{-1} (Δ^{22} trans double bond⁷). The $^1\text{H-NMR}$ spectrum (90 MHz; CDCl_3) of compound III showed 52 protons. An unresolved triplet at δ 5.0 (2H) indicated two vinylic protons with adjacent- CH_2 -group; a triplet at δ 4.3 (1H) indicated C-3 proton; a singlet at δ 1.9 (3H) suggested an acetyl group while the methyl protons appeared between δ 0.7 to 1.0 (24H) suggested a tetracyclic triterpene⁸. The rest of the methylene and methine protons appeared between δ 1.2 and 1.6 (22H). In the mass spectrum of this compound the molecular ion M^+ appeared at m/z 468 while other important fragment ions at m/z 453 ($M-\text{CH}_3$); 425 ($M-\text{C}_3\text{H}_7$); 408 ($M-\text{CH}_3\text{COOH}$); 393 ($M-\text{CH}_3\text{COOH}-\text{CH}_3$); 357 ($M-\text{C}_8\text{H}_{15}$); 289 ($M-179$); 218 ($M-250$); 203 ($M-265$) were also observed.

Hydrolysis of compound III with 1% alcoholic KOH yielded an alcohol mp 176–8°; $C_{30}H_{50}O$ M^+ m/z 426. The alcohol gave positive Liebermann-Burchardt test⁶ for triterpene and also indicated in its IR spectrum (KBr) a band for hydroxyl function at 3300 cm^{-1} and absence of the acetate carbonyl at 1730 cm^{-1} .

From the above analytical, spectral and chemical evidence compound III and the alcohol obtained from it were identical with gluanol acetate (acetate of 13α , 14β , $17\beta(\text{H})$, $20\alpha(\text{H})$ -lanosta-8, 22-diene- 3β -ol) and gluanol respectively. Gluanol acetate was previously isolated by Chowdhary and Sen⁹ from *Ficus glomerata* Roxb. leaves while gluanol was isolated by Merchant *et al*¹⁰ from *F. glomerata* Roxb. fruits. These compounds could not be directly compared due to non-availability of the respective authentic samples.

It is significant that this is the second report of isolation of gluanol acetate even though *n*-triacontranol, β -amyirin and their acetates are prolific in nature. The isolation of gluanol acetate from *F. hispida* Linn may be of chemotaxonomical interest.

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A TECHNIQUE FOR PRESERVATION OF MICROBIAL CULTURES SUITABLE FOR SMALL QUALITY CONTROL LABORATORIES

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LYOPHILIZATION; although the most reliable method for preservation of bacterial culture for a long time is not useful in small laboratories, especially as it is expensive. A method which elaborates the one suggested earlier by Lederberg (cited in Hunt *et al*¹) is described in this note which could prove useful for small quality control laboratories. A suspending medium (composition: yeast extract 0.4%, lactose 2%, gelatin 1%, ascorbic acid 0.25% pH 6.2 ± 0.2 ; sterilised at 15 p.s.i for 15 min) has been developed which was not reported earlier. The survival of different microorganisms including lactobacilli and yeast has been studied after drying them on bead.

Porcelain beads (ht-4 mm \times diam-3 mm approx; used for insulation of electrical wires) were boiled with concentrated HCl and washed till they were free from acid. The beads were heated to red heat for 1 hr in a porcelain basin to remove inhibitors and cooled. Five or ten such beads were introduced in 75 mm \times 10 mm or 50 mm \times 10 mm borosilicate test tubes respectively and cotton-plugged and sterilised at 160° for 2 hr.

A thick suspension was made by using suspending media from fresh slant cultures of bacterial strains and 72 hr cultures of yeast strains. For lactobacilli cell the mass was separated by centrifugation before preparing the suspension. One or two drops of the suspension were transferred to the tubes containing the beads. The tubes were rotated to smear the suspension on beads and then kept in a vacuum desiccator containing silica gel, at 6–8°C. For survival test, deMan Rogosa-Sharpe medium², soyabean casein digest and antibiotic assay medium³—I were used respectively for lactobacilli, yeast and other cultures. Important characteristic tests were carried out to check the identity of viable cultures. A total of 40 different strains were studied. The cultures: *Staphylococcus aureus*, *S. epidermidis*, *Salmonella typhi*, *S. typhimurium*, *Sarcina lutea*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Bordetella bronchiseptica*, *Saccharomyces cerevisiae*, *Candida albicans*, *Micrococcus glutamicus*, *M. flavus*, *Lactobacillus plantarum* (Strains: Rbt; CW; ATCC8014), *L. acidophilus* (Strains: Bf; Rt; Pg), *L. leichmannii*, *Streptococcus faecalis* survived for the whole period of study i.e. 12.5 months. *L. helveticus* survived 7.5 months and another strains of *L. helveticus*-Gp and *L. salivarius*-Dg survived only for 5 months.

In the present suspending medium gelatin was used to provide a protective layer on the microbial cell and ascorbic acid was employed as an antioxidant. In this experiment other than *L. leichmannii*, *L. plantarum* (ATCC8014) and *S. faecalis* all the lactobacilli strains were of intestinal origin and two of them (*L. acidophilus* CA and HA) did not grow even after two and a half months. These strains were anaerobic. Possibly drying on beads deprived them of anaerobic environment.

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MODIFICATION OF SEX EXPRESSION IN MULBERRY BY COLCHICINE

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In flowering plants, regulation of sex expression has been attributed to genetic, environmental and chemical factors¹. Induction of flowers with opposite sex or mixed type of sexes has been reported by several workers both in monoecious and dioecious plants using growth regulators², chemicals of morphactin group^{3,4} and certain ions⁵. However, there is no report on the modification of sex expression in these plants by colchicine.

The present observation is an offshoot of a project designed to improve the nutritive quality of leaves by inducing tetraploidy and subsequently evolving triploids by hybridization with different desirable diploid strains. When buds of female mulberry variety Kanva-2 started sprouting, they were treated with 0.4% aqueous solution of colchicine (Loba, India) prepared in 5% glycerine, for 8 hr for three consecutive days. The buds were wrapped with a piece of cotton wool, and colchicine solution was given from a glass dropper from time to time to keep it moist. After the treatment, cotton wool was removed and the buds were washed with distilled water. Six buds treated in 5 replications. Controls received only distilled water treatment. Healthy female inflorescences were produced by control buds (figure 1). Immediate effects of colchicine treatment were suppression of female inflorescences, production of mixed type of inflorescences and male inflorescences (figures 2–4). The frequency of production of male (43.27%) and mixed type of inflorescences (25.96%) was higher than that of suppressed (23.08%) and healthy female (7.69%) inflorescences. Pollen fertility was 86% as determined by staining with 2% acetocarmine in glycerine solution.

Sex expression is controlled by the optimal balance between endogenous auxin and gibberellin¹ and/or between gibberellin and ethylene⁶. Auxin² and ethylene⁷ promote femaleness and gibberellin⁵ favours maleness in dioecious plants. Decrease in auxin/ethylene level and or increase in the level of gibberellin induces the development of male flowers on female plants and *vice versa*¹.

In the opinion of the present authors the modification of sex expression in female plants of *Morus alba* L. Var Kanva-2 is either due to the imbalance between