ISOLATION OF SISTOSTEROL FROM SUGARCANE WASTE AND ITS BIOCONVERSION INTO ADD USING *ARTHROBACTER OXYDANS*.

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THE waste material of sugar industries, pressed mud, has been explored as an unconventional source of sterols to be used for steroidal drugs after its microbiological conversion into ADD (Androsta 1,4-diene, 3,17-dione). Chemical oxidation[1] of sterols into ADD has been found to be uneconomical, but the side-chain of sterols at C–17 can be selectively cleaved to yield ADD by micro-organisms[2]. A bacterium *Arthrobacter oxydans*, has been isolated[3] which converts sugarcane sterols into 17-ketosteroids like ADD in the presence of metabolic inhibitors.

Sugarcane sterol was isolated from the soft wax fraction of dried pressed mud by the method of Bose and Gupta[4] with certain modifications. The purity of sterol was 94% by Libberman Burchard reaction[5]. The medium on which the bacterium was grown contained (g/l) $NaHPO_4$. $2H_2O$, 3:KH_2PO_4, 3; NaCl, 0.2; $(NH_4)_2SO_4$, 1; $MgSO_4$. $7H_2O$,0.3; $CaCl_2$. $2H_2O$,0.1; $FeSO_4$. $7H_2O$,0.001; sodium molybdate, 0.0001. The pH was adjusted to 7.2 and fine suspension of sistosterol (0.1%) was made in the above medium. The bacterium was allowed to grow in a 250 ml flask containing 50 ml of the medium on a reciprocal shaker (50 strokes/min). Sistosterol consumption, growth of the bacterium and 17-ketosteroid accumulation in the culture broth in the presence and absence of metabolic inhibitors (α, α-dipyridyl and sodium arsenite) were estimated. To detect the metabolite in the culture broth, an ethyl acetate extract was subjected to TLC and the spots developed on exposure to iodine vapour were marked. Preparative TLC was performed in benzene:ethylacetate (5:1) to obtain desired metabolite which was recrystallized. The m.p. was determined. The metabolite was further identified by UV, mass, IR and PMR spectra and compared with those obtained using authentic sample.

The bacterium started growing after 12 hr and reached to maximal after 36 hr (figure 1). In the presence of inhibitors (α, α-dipyridyl and sodium arsenite) the accumulation of 17-ketosteroid in the culture broth took place after 18 hr (figure 2). These inhibitors protect the steroid nucleus from being used up by the bacterium, but the consumption of the side-chain at C–17 position continued. As a result

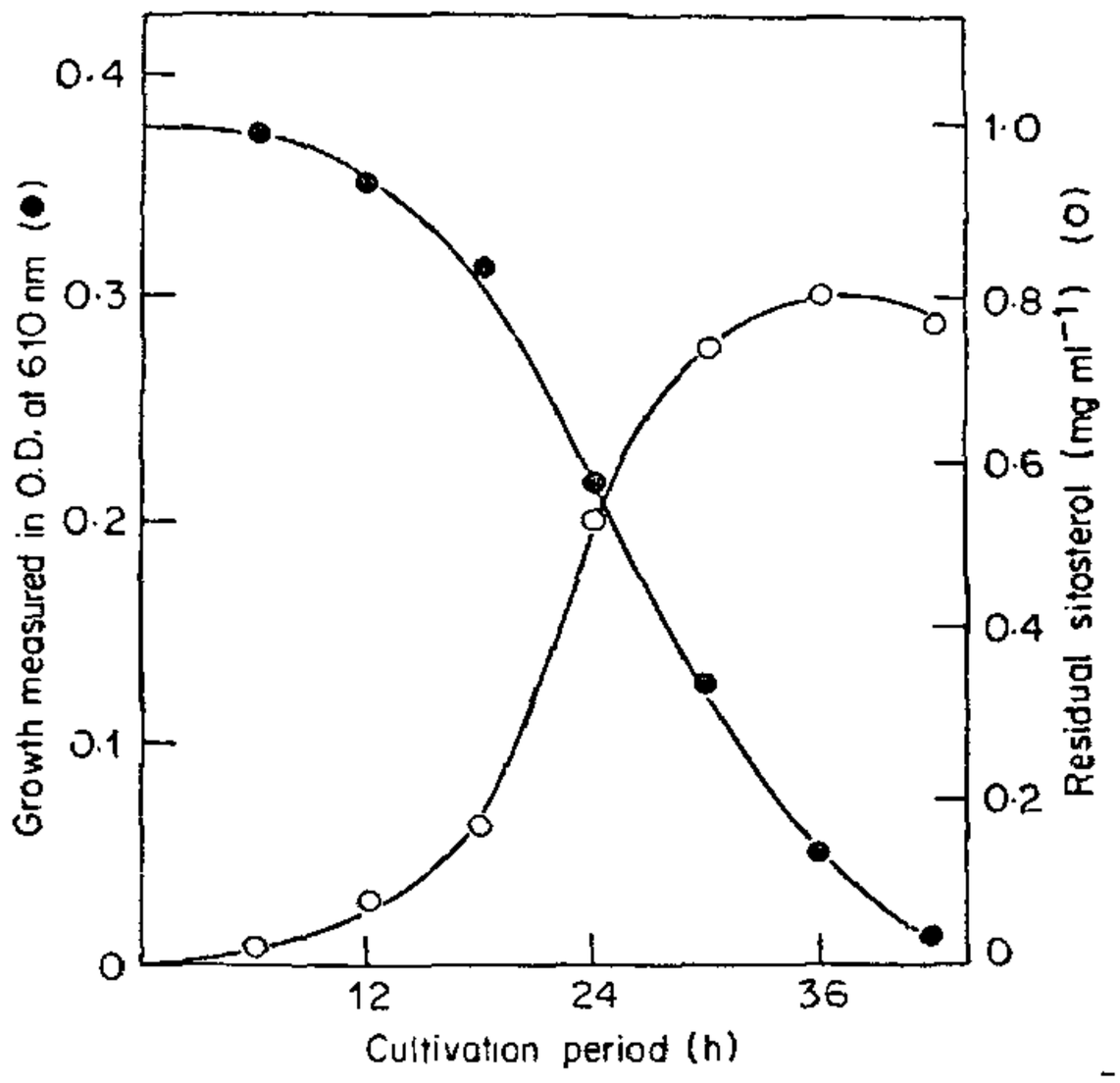

Figure 1. *Arthrobacter oxydans*: Growth (open circle) and sterol consumption (closed circle) with respect to time. Temperature 32° C and pH 7.2.

17-ketosteroids got accumulated in the culture broth. On TLC the intense brown colour under iodine was noticed with R_f value of 0.46. This compound was further identified and the data obtained were as follows: m.p. 141 C; M. W. 286 (mass spectra); the compound showed λ_{max}^{EtOH} at 243 nm; IR (as KBr pellets): 1738 cm^{-1} (17–C=0); 1665 cm^{-1}, 1626 cm^{-1} and 1604 cm^{-1} ($\Delta^{1,4}$–3–C=0); PMR (in $CDCl_2$) showed peaks at δ 0.95 (C_{18}–CH_3), 1.26 (C_{19}–CH_3). The compound was

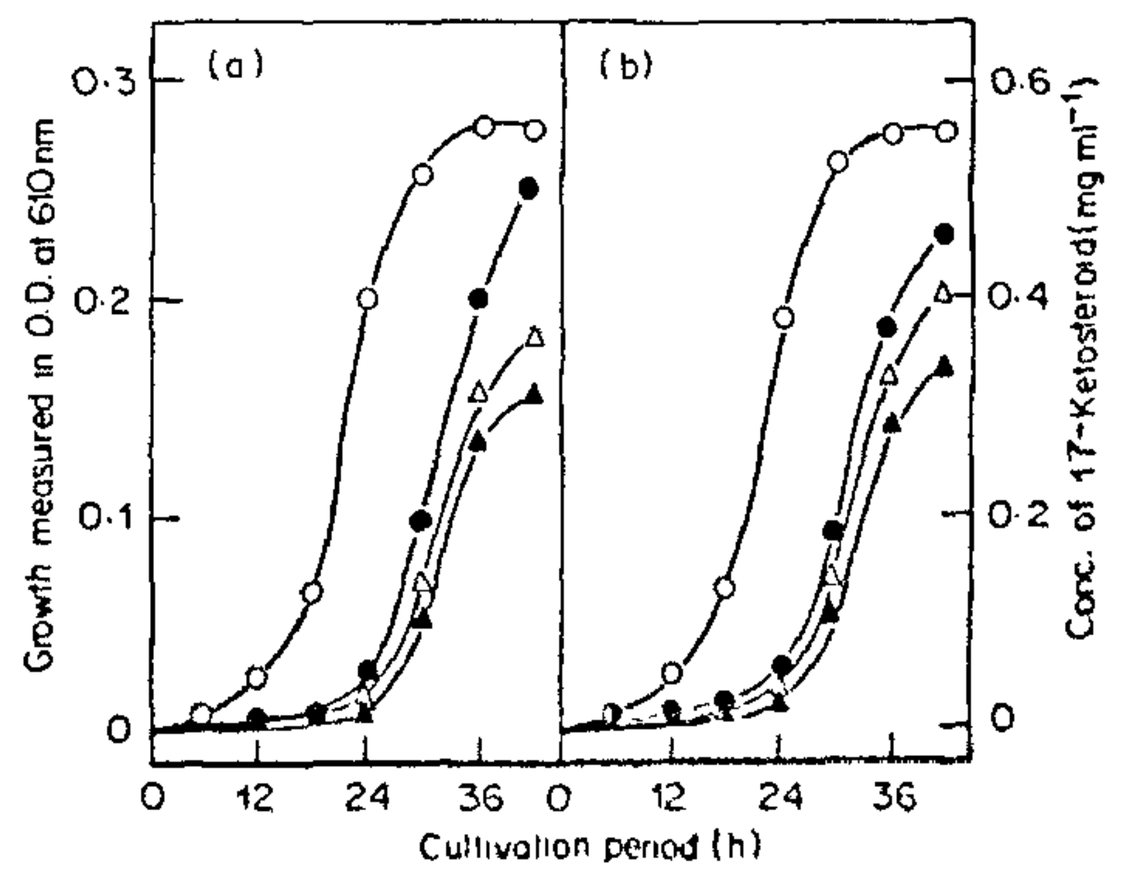

Figure 2. *Arthrobacter oxydans*: Growth (open circle) and 17-ketosteroid accumulation in the culture broth after the addition of metabolic inhibitors, α,α-dipyridyl (2a) [(closed triangle) 0.05%; (closed circle) 0.10%; (open triangle) 0.15%], and sodium arsenite (2b).

identified as androsta 1,4-diene-3, 17-dione (ADD). This was further confirmed by the superimposable spectra of the authentic sample (Sigma Chemical, St. Louis, U.S.A). For the first time sugarcane sterols have been microbiologically converted into ADD using *Arthrobacter oxydans*. There are reports dealing with sterol degradation[6] and pregnenolone utilization[7] using *Arthrobacter simplex*, but no reports appear to be available on sterol utilization by *A. oxydans*. This is a new species having high efficiency to convert sterols into ADD (figure 2). Other workers using *A. simplex* reported only 30% conversion of sterols into ADD[6], whereas in the present study 50% conversion of sterols has occurred. At present ADD is obtained from solasodine and diosagenin which have limited availability. ADD is used for the synthesis of steroidal drugs. Hence, the present work attempts to utilize a waste material into valuable products using a new high efficiency bacterial species—*Arthrobacter oxydans* not reported earlier for its capability to utilize sterols.

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DISTRIBUTION OF CANAVANINE IN SOME INDIAN GALEGEAE (FABACEAE) AND ITS SYSTEMATIC SIGNIFICANCE

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CANAVANINE, first detected in *Canavalia ensiformis* DC., is the most characteristic free non-protein amino acid of the Fabaceae. Canavanine occurs in the seeds of about 35% of the 150 genera and 60% of the 540 species of the Fabaceae[1-3]. The present account deals with the distribution of canavanine in 48 Indian samples of 15 species of *Indigofera, Tephrosia, Millettia, Mundulea* and *Gliricidia* falling into three subtribes of Galegeae[4] and its systematic significance.

The following samples were studied:

A. Subtribe Indigofereae: 1. *Indigofera astragalina* DC., (Bannerghatta); 2. *I. nummulariifolia* (L.) Livera ex Alston (Bannerghatta); 3. *I. linnaei* Ali (Bannerghatta, Bangalore).

B. Subtribe Tephrosieae: 4. *Tephrosia purpurea* (L.) Pers., (Bhubaneshwar, Cuttack, Konark, Waltair, giant form from Waltair, Anakapalli, Bojjannakonda, Araku valley, Vijayawada, Madras, Rameswaram, Kovalam, Trivandrum, Malpe, Dona Poula, Markhandi-Udadoan, Bannerghatta, Savanadurga, Bangalore); 5. *T. maxima* (L.) Pers., (Waltair Bojjannakonda, Kovalam); 6. *T. candida* DC., (Cuttack, Waltair); 7. *T. hamiltonii* J. R. Drumm., (Patna); 8. *T. villosa* (L.) Pers., (Waltair, Bannerghatta, Savanadurga); 9. *T. calophylla* Bedd., (Bannerghatta); 10. *T. tinctoria* Pers., (Bannerghatta); 11. *T. pumila* (Lam.) Pers., (Waltair, Dharwar); 12. *T. noctiflora* Bojer ex Baker (Cuttack); 13. *Millettia peguensis* Ali (Bangalore); 14. *Mundulea sericea* (Willd.) A. Chev., (Waltair, two samples). C. Subtribe Robinieae: 15. *Gliricidia sepium* (Jacq.) Kunth ex Walp., (Waltair, Bangalore, three populations each).

Canavanine was extracted from the seeds with 1N HCl and was co-chromatographed on paper with L-canavanine (Sigma Chemicals, U.S.A) Pentacyanoammonioferrate (0.25% w/v) was used to detect canavanine which was indicated by magenta colour[1,5].

None of the species of *Indigofera* and *Tephrosia* studied here and elsewhere so far contained canavanine. In *Mundulea sericea* canavanine was found in the present work but there were one negative[1] and two positive reports[2,3] for this species. Only the Waltair samples of *Gliricidia sepium* contained canavanine while the Bangalore samples were devoid of it. A similar situation exists in *Abrus precatorius* (tribe Vicieae), *Lespadeza tomentosa* and *Hippocrepis comosa* (both of tribe Hedysareae)[3].

Previous work indicates that canavanine was irregularly distributed in the Galegeae[3]. Of the seven subtribes delimited by Bentham[4], only subtribe Robinieae consistently contained canavanine while the subtribes Psoralieae, Indigofereae and Brongniartieae were consistently devoid of it[3]. In the subtribes Tephrosieae, Coluteae and Astragaleae, canavanine irregularly distributed. The present results are in general agreement with this situation. A pattern similar to that of the Galegeae exists in the tribes Podalyrieae, Genisteae, Hedysareae, Dalbergieae, Vicieae and Phaseoleae of the Fabaceae[3].