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STRUCTURE OF PHELLAMURIN

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So far the structure of phellamurin[1] has been thought to be 3,5,7,4′-tetrahydroxy-8-(γ-hydroxyisovaleryl)-flavanone-7-*O*-glucoside, although the aglycone expected from above structure had not been obtained When phellamurin was hydrolyzed with acid, phellamuretin (3,5,4′-trihydroxy-(6″,6″-dimethyldihydropyrano)-2″,3″ 7,8-flavanone) was obtained as an aglycone During the study on the degradation of phellamurin by *Aspergillus niger*, a colourless crystalline compound neophellamuretin (m p 190°) having properties of a flavanonol was isolated from the ether extract of culture medium The properties of this compound are not identical with that of phellamuretin

An ethanolic solution of this compound gave a purplish brown colouration with $FeCl_3$ When reduced with Mg or Zn powder and conc HCl, a reddish purple colouration was developed, characteristic of flavanonols [2] The aglycone also shows UV absorption peaks (in EtOH) at 300 and 340 nm, the former peak undergoes the bathchromic shift of 20 nm on the addition of $AlCl_3$ [3]

Neophellamuretin coincides in all its properties with an aglycone of phellamurin obtained by hydrolysis with β-glucosidase Acid treatment of neophellamuretin gives phellamuretin identical with an authentic sample by PC, TLC, spectrophotometry and m m p MS of neophellamuretin shows a parent ion peak at *m/e* 356

The PMR spectrum (60 MHz, $CDCl_3$) of neophellamuretin acetate exhibits the *AB* system of H-2 and H-3 at 5 40 ppm (*d*) and 5 74 (*d*, $J_{2,3}$ 12 5 Hz) [4,5] A signal at 3 24 shows methylene protons of benzyl structure which are split into doublet (*J* 7 Hz) by coupling to the next methine proton as a triplet (*J* 7 Hz) at 5 05 The signals at 1 54 and 1 68 show methyl groups[5] (3H each, singlet) which have a long range coupling with the methine proton at 5 05 The above results reveal that an isoprenyl group in the structure of neophellamuretin The three methyl groups at 2 31 (6H) and 2 36 (3H) are due to aromatic acetyl groups One acetyl group derived from C-3 hydroxyl group is 2 02 ppm The proton at 6 61 (singlet) is a signal of H-6 (A ring) and B ring is monosubstituted at the 4 position, 7 16 and 7 49 ppm for the H-2′,6′ and H-3′,5′ protons IR spectrum of neophellamuretin acetate (Nujol) has no signal due to a tertiary alcohol as expected from the structure of phellamurin

From the above results, neophellamuretin is 3,5,7,4′-tetrahydroxy-8-isoprenylflavanone, and the structure of phellamurin should be the corresponding 7-*O*-glucoside

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EXPERIMENTAL

Chemicals Phellamurin has been isolated from the leaves of *Phellodendron amurense* by the method of Hasegawa and Shirato [1]

Culture Stock culture of *Aspergillus niger* was maintained on agar slants The growth medium was the modified Czapek-Dox medium with some microelements ($FeCl_3$ $6H_2O$, 20 mg $ZnSO_4$ $7H_2O$ 10 mg $MnSO_4$ $4H_2O$ 3 mg Na_2MoO_4 $2H_2O$ 1 5 mg $CuSO_4$ $5H_2O$ 1 mg) glucose 20 g and phellamurin 0 1 g and its pH was adjusted to 4 5 with HCl The soln of phellamurin and remaining ingredients were sterilized separately and combined aseptically in the flasks prior to inoculation 1 l of the liquid culture medium was inoculated with spores grown on 5 slants and incubated for 4 11 days at 25

Isolation 2 l of liquid medium were filtered and extracted with Et_2O After removal of Et_2O the remaining mass was dissolved in EtOH and applied to a column of polyamide The column was eluted successively with 100 ml each of 0 20 40, 60 80 100% aq EtOH The fractions eluted with 60 and 80% EtOH were concentrated and examined by TLC on silica gel plates with a solvent $CHCl_3$ EtOAc HCOOH (5 4 1) Neophellamuretin R_f 0 8) was isolated from silica gel plates with EtOH and recrystallized from EtOH H_2O

Neophellamuretin From EtOH m p 189 190 (Found C 67 30 H 5 73 $C_{20}H_{20}O_6$ requires C 67 41 H 5 61%) *m/e* 356 (25%) Acetate m p 125 126 (from dil EtOH) ν_{max}^{Nujol} 2860 2833 1760 1690 1610 1370 (δ) cm^{-1}

Hydrolysis of phellamurin Phellamurin (0 1 g) was hydrolyzed with 0 1 g β-glucosidase The aglycone neophellamuretin was extracted with Et_2O and recrystallized from EtOH m p 190 Sugar was determined as glucose with PC

Acid treatment Neophellamuretin (20 mg) was heated in 5% H_2SO_4 added with small vol of EtOH at 100 for 3 hr After evaporation of EtOH the solution was extracted with Et_2O phellamuretin obtained was recrystallized from EtOH m p 221 Acetate m p 199 NMR ppm 1 33 (6H s gem-dimethyl) 1 68 and 2 59 (2H each *t J* 7 Hz H-5′ and H-4″), 1 99 (3H s aliphatic acetyl group) 2 30 and 2 34 (3H each s aromatic acetyl group) 5 35 and 5 56 (1H each *d J* 12 Hz H-2 and H-3) 6 22 (1H, s H-6) 7 15 (2H *d J* 9 Hz H-3 5) 7 44 (2H *d J* 9 Hz H-2 6)

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ALKALOIDS OF *DATURA DISCOLOR*

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Plant *Datura discolor* Bernh A species native to the desert regions of south-eastern California and Mexico, sometimes confused with *D meteloides* D C which is found in similar locations but may be distinguished from the latter species by the presence of 5 purple flushes in the throat of the corolla and by its pentagonal calyx and characteristic seeds Taxonomically it accords with Safford's section Dutra[1] of the genus *Previous work* Pharmacognostical description assay of total alkaloids in morphological parts and characterization of hyoscine as principal alkaloid [2] Ontogenetic production of total alkaloids of

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