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PULCHELLIDINE, A NOVEL SESQUITERPENE ALKALOID ISOLATED FROM GAILLARDIA PULCHELLA FOUG.

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So far Gaillardia pulchella Foug. has been found to elaborate "abnormal" sesquiterpene lactones, pulchellin(1), pulchellin B, C and D(2) except a guaianolide, gaillardin(3), and to be of a significant infraspecific variation with the locale of the native plants. We now wish to report the isolation and structure determination of a new sesquiterpene lactone alkaloid, pulchellidine(I), from the same plant collected near St. Augustin, Florida, which has not been isolated earlier from several genera of compositae, and found to exhibit an antiinflammatory activity[*2].

The chloroform extract of the air dried overstem material yielded, with benzene-chloroform elutions on chromatography over neutral alumina, mainly a new basic constituent. After separation of a sticky minute contaminant[*3] by extensive fractional crystallizations from several different solvent systems, pulchellidine was isolated pure. The following elution with chloroform containing methanol afforded a minor non-basic constituent referring to Compound H: $C_{12}H_{18}O_3$[*4]; mp 225-228°; $(\alpha)_D^{18^\circ}$ -66.0°(c,1.0; EtOH).

Pulchellidine: $C_{20}H_{33}O_4N$; M.W. 351.241 by Mass. (M^+, 351.243); mp 185-186°(Me_2CO-Et_2O); $(\alpha)_D^{17.5^\circ}$ -22.5°(c,1.33; EtOH); IR[*5] ν 3350(hydroxy), 1774(γ-lactone); NMR[*6] δ 0.80 s (≥C-C$\underline{H}_3$), 1.25 d,6 (>CH-C$\underline{H}_3$), 3.65 d,5 (>C$\underline{H}$-OH), 4.15 m (>C$\underline{H}$-OH and >C-C-CO-O-C$\underline{H}$-); UV end absorption; GLC Rt 8.5[br]; 9.5 for TMS derivative on SE-30: TLC R_f 0.54 [Silica Gel, Me_2CO-EtOH(1:1)].

Acetylation with Ac_2O/pyridine gave the diacetate: $C_{24}H_{37}O_6N\cdot\frac{1}{2}H_2O$; M.W. 435.262 by Mass. (M^+, 435.259); mp 135-136°; $(\alpha)_D^{27^\circ}$ -24.0°(c,1.0; $CHCl_3$); IR ν 1780, 1760, 1728; NMR δ 0.98 s (≥C-

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*2 Presented at the 2nd International Symposium on Pharmaceutical Chemistry held in Münster, July 22-26, 1968, paper abstract 1-13.

*3 A little bit higher R_f spot(0.57) on TLC observed with crude pulchellidine has been proved equivallent to its isomer, neopulchellidine: mp 131-134°; $(\alpha)_D^{21^\circ}$ -13.0°(c,1.0; $CHCl_3$).

*4 Satisfactory elementary analyses were obtained for all new compounds by Mrs. K. Hirose of our laboratory to whom we are grateful.

*5 IR-spectra were taken in KBr pellet and the band positions are expressed by cm^{-1}.

*6 NMR-spectra were determined at 60 Mc in $CDCl_3$ (internal TMS), and chemical shifts are reported in ppm (δ) and coupling constants are cited in cps. Abbreviations: s=singlet, d=doublet, t=triplet, m=multiplet and c=complex.

CH_3), 1.01 d,6 (>CH-CH_3), 4.13 t,8 (>C-C-CO-O-CH_2-), 4.65 d,5 (>CH-OAc), 4.93 t,9 (>CH-OAc).

Lithium aluminium hydride reduction(THF, r.t., 20 hr.) afforded the tetraol(II): $C_{20}H_{37}O_4N$; mp 103-105°; $(\alpha)_D^{13°}$ -9.6°(c,1.0; EtOH); NMR δ 0.70 s (>C-CH_3), 1.19 d,6 (>CH-CH_3), 1.26-2.31(16H), 2.31-2.91 m [$N(CH_2-)_3$], 3.48 m (>CH-OH and -CH_2-OH), 4.01 m (2 x >CH-OH), 4.57 s (3 x >CH-OH and -CH_2-OH; disappeared by addition of D_2O), which was subjected to dehydrogenation in a sealed tube (15%-Pd/C, N_2, 300°, 10 min.) to yield S-guaiazulene (2.8%), linderazulene (0.4%) and pyridine (15.6%). UV-spectra of the azulenes and IR-spectra of the azulene trinitrobenzene complexes were all identical with those of the authentic specimens*7. Thus the unique pseudoguaiane carbobicyclic skeleton in pulchellidine was particularly established by such a poor yield of the azulenes. In addition, the formation of linderazulene suggests that the lactone ether oxygen of pulchellidine must be linked to the carbon atom, not neighboring to the quaternary carbon, whose situation reflexes on the broadened triplet signal at about 4.15 ppm of the questioned proton in pulchellidine and its diacetate. Furthermore, the liberation of pyridine would indicate the presence of piperidine moiety in pulchellidine.

On further inspection of NMR-spectra of pulchellidine, its acetate and the tetraacetate of the tetraol [$(\alpha)_D^{14°}$ +8.0°(c,0.7; EtOH)], a broad signal of N-methylenes centred around 2.5 ppm for nearly six protons causes a downward shift by about 1 ppm in pulchellidine hydrochloride (mp 205-208°) and hydrobromide (mp 208-210°). The presence of $N(CH_2-)_3$ group in pulchellidine was further evidenced by prominent fragment ion peaks at 98.097 and 84.085 corresponding to the cations of N-methylenepiperidine ($C_6H_{12}N$, m/e 98.097) and piperideine ($C_5H_{10}N$, m/e 84.085), respectively, in the Mass spectra of pulchellidine and its acetate. It is more likely that N-piperidinomethyl group should be linked to α-carbon atom of γ-lactone in pulchellidine by comparison of pKa' valuses for pulchellidine (9.2), pulchellin*8(7.5), piperidine (10.5) and ethyl α-(N-piperidino)-methylpropionate*9(9.0). This assumption on the basis of the forgoing physico-chemical data was further concreted by quantitative degradation of its methiodide ($C_{21}H_{36}O_4NI$; mp 136-137°; IR ν 3490, 1765, 1734) with hot dilute Na_2CO_3(100°, 15 min.) to an α,β-unsaturated γ-lactone consisting of a carbobicyclic sesquiterpene named epipulchellin (III) ($C_{15}H_{22}O_4$) under liberation of N-methylpiperidine ($C_6H_{13}N$). The pure sample of the degradation product shows no depression of melting point on admixture of pulchellin*8. It exhibits almost identical IR-spectrum with that

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*7 We thank to Drs. K. Takeda and H. Minato of Shionogi Pharmaceutical Co. Ltd. for gift of these samples.

*8 The sample was donated by Dr. W. Herz of F.S.U. to whom we are due. mp 165-168°; $(\alpha)_D^{26°}$ -36.21°(c,2.43; $CHCl_3$); diacetate: mp 123-125°; $(\alpha)_D^{25°}$ -28.96°(c,1.83; $CHCl_3$) (1).

*9 Prepared according to C. A. Weisel, R. B. Taylor, H. S. Mosher and F. C. Whitmore: J. Am. Chem. Soc. 67, 1070 (1945).

of pulchellin, but only slightly differs at the peaks of C=O, C=C and C-O streching (1757, 1670, 1124^{S} and 1748, 1659, 1126^{W}), and methyl (1352^{W} and 1357^{S}) bending vibrations (Fig. 1).

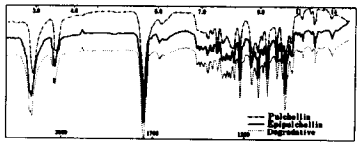


Fig. 1

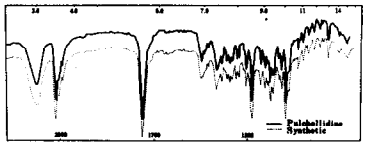
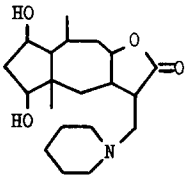
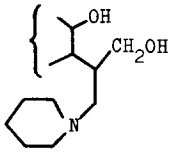


Fig. 2

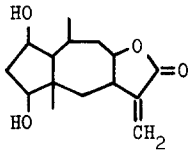
Epipulchellin: mp 162-164°; $(\alpha)_D^{25°}$ -43.3°(c,0.65; $CHCl_3$); UV λ_{max}^{EtOH} 208 mμ (ε 9725) ($\Delta^{\alpha,\beta}$-γ-lactone); IR ν 3486, 3442 (hydroxy), 1757, 1226 (γ-lactone), 1670 (double bond); NMR δ 0.87 s (≥C-C$\underline{H}_3$), 1.23 d,5 (≥CH-C$\underline{H}_3$), 3.62 d,4 (≥C$\underline{H}$-OH), 4.10 c (≥$\overline{C\text{-}C\text{-}CO\text{-}O\text{-}C\underline{H}}$-), 5.47 d,3; 6.14 d,3 (≥C=C$\underline{H}_2$). Diacetate: $C_{19}H_{26}O_6$, mp 128.5-129.5°; $(\alpha)_D^{20°}$ -58.7°(c,0.46; $CHCl_3$); IR ν 1774, 1741, 1238; NMR δ 0.98 s (≥C-C$\underline{H}_3$), 1.05 d,7 (≥CH-C$\underline{H}_3$), 2.03 s (-OCOC$\underline{H}_3$), 2.09 s (-OCOC$\underline{H}_3$), 4.13 c (≥$\overline{C\text{-}C\text{-}CO\text{-}O\text{-}C\underline{H}}$-), 4.78 d,5 (≥C$\underline{H}$-OAc), 5.0 c (≥C$\underline{H}$-OAc), 5.40 d,3; 6.17 d,3 (≥C=C$\underline{H}_2$). Sulfite: $C_{15}H_{20}O_5S$; mp 167-168°(dec.); IR ν 1774(γ-lactone), 1198(sulfite). There also exists no significant difference between the NMR-spectra of epipulchellin and pulchellin except the chemical shifts of tertiary (0.88 and 0.90) and secondary (1.23 and 1.37) methyl protons. These spectral data and the following chemical transformation of pulchellidine(I) to dihydroanhydro-dehydro-dihydroepipulchellin(VII) suggest that epipulchellin must evidently be a stereoisomer, but not a structural isomer of pulchellin.



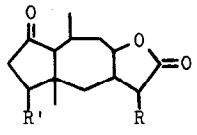
I



II



III



IV	R, $-CH_2NC_5H_{10}$	R', OH
V	R, $=CH_2$	R', OH
VI	R, $=CH_2$	R', H
VII	R, $-CH_3$	R', H

Oxidation of pulchellidine with CrO_3/HOAc afforded dehydropulchellidine(IV): $C_{20}H_{31}O_4N$; mp 117.5-119°(ether-petropleum ether); $(\alpha)_D^{18°}$ +28.8°(c,0.87; EtOH); IR ν 3400(hydroxy), 1770(γ-lactone), 1739(cyclopentanone); NMR δ 0.88 s (≥C-C$\underline{H}_3$), 1.44 d,6 (≥CH-C$\underline{H}_3$), 3.92 d,4.5; d,2.5 (≥C$\underline{H}$-OH), 3.95 c (≥$\overline{C\text{-}C\text{-}CO\text{-}O\text{-}C\underline{H}}$-), which was degradated by treatment of its methiodide with dilute Na_2CO_3 to dehydroepipulchellin(V); $C_{15}H_{20}O_4$; mp 188-190°; IR ν 3474(hydroxy), 1762(γ-lactone), 1727(cyclopentanone), 1673(double bond); UV λ_{max}^{EtOH} 209 mμ (ε 11304) ($\Delta^{\alpha,\beta}$-γ-lactone). Dehydration with mesylchloride and hot pyridine gave anhydrodehydroepipulchellin(VI, $\Delta^{3,4}$): $C_{15}H_{18}O_3$;

mp 231-234°; $(\alpha)_D^{22°}$ -9.7°(c,0.155; $CHCl_3$); IR ν 1759, 1663^{sh}($\Delta^{\alpha,\beta}$-γ-lactone), 1706, 1671 (cyclopentenone); UV λ_{max}^{EtOH} 212.5 mμ (ε 11640); NMR δ 1.36 s (≥C-C$\underline{H}_3$), 1.46 d,6 (≥CH-C$\underline{H}_3$), 3.80 m (≥C-C-CO-O-C$\underline{H}$-), 5.36 d,3; 6.14 d,3 (≥C=C$\underline{H}_2$), 6.18 d,6; 7.23 d,6 (-C$\underline{H}$=C$\underline{H}$-CO-), which was hydrogenated with 5%-Pd/C to give rise to the cyclopentanone derivative (VII): $C_{15}H_{22}O_3$; mp 146-148°; $(\alpha)_D^{21°}$ -125°(c,1.0; $CHCl_3$); IR ν 1776(γ-lactone), 1730(cyclopentanone); NMR 1.27 s (≥C-C$\underline{H}_3$), 1.33 d,6 (≥CH-C$\underline{H}_3$), 1.16 d,7 (≥CH-C$\underline{H}_3$), 3.75 c (≥C-C-CO-O-C$\underline{H}$-).

Thus 1, 3-diol and lactonic hydroxy group of pulchellidine are located at C_2, C_4 and C_8 of a pseudoguaiane skeleton, and piperidinomethyl group are linked to C_{11} again. This was further substantiated by the partial synthesis of pulchellidine(I) by addition of piperidine to epipulchellin(III) (r.t., N_2, 2 days). The Michael addult(mp 185-186° from $CHCl_3$-petroleum ether) was proved exactly identical with pulchellidine by determination of mixed melting point, comparison of TLC, GLC and IR-spectra (Fig. 2). The structure of pulchellidine(I) was completely established by both degradative and synthetic way.

The stereochemical studies of pulchellidine and epipulchellin mentioned above as well as pulchellin(1) are now in progress along with new stereoisomers, neopulchellidine and neopulchellin isolated from another source, which will be reported in the following paper.

It is noteworthy to allude that pulchellidine should be biologically formed in the plant by a Michael addition of piperidine or its congener to α,β-unsaturated γ-lactone or the equivalents of epipulchellin. The piperidine nucleus would possibly be originated from such amino acids as lysine, but not from a polyacetyl precursor, like in biosyntheses of sedamine(4) and N-methylisopelletierine(5).

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