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CONSTITUENTS OF *GAILLARDIA* SPECIES—II

STRUCTURES OF PULCHELLIN B AND PULCHELLIN C[1]

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Abstract—A collection of *Gaillardia pulchella* Foug. from New Mexico furnished three new sesquiterpene lactones, pulchellin B, C and D, instead of pulchellin previously isolated from the Southeastern variety. Structures of pulchellin B and C were established. Helenalin was isolated from *Gaillardia multiceps* Greene and *Gaillardia megapotamica* (Spreng.) Baker.

In an earlier paper[1] the isolation and structure of pulchellin (I), the main sesquiterpene lactone constituent of *Gaillardia pulchella* Foug., was reported as part of our inquiry into the chemistry of *Helenium* and related species. Collections of *G. pulchella* used in the earlier work represented South-eastern races which are morphologically and cytologically identical with races found on the Gulf coast of Texas and Louisiana, but differ in these respects from races farther West.[2] This is now borne out chemically as well by an examination of a *G. pulchella* collection from Eastern New Mexico which did not furnish pulchellin, but three new sesquiterpene lactones named for convenience pulchellin B, C and D.[3,4] Structures of pulchellin B and C have been determined and are reported in this paper.

Pulchellin B(III), $C_{17}H_{22}O_5$, m.p. 215–218°, $[\alpha]_D^{27°}$ +92·7°, and pulchellin C(II), $C_{15}H_{20}O_4$, m.p. 199–202°, $[\alpha]_D^{27°}$ +125°, were obviously α,β-unsaturated lactones ($\lambda_{B\ max}$ 210 and 325 mμ, ε_B 8830 and 53, $\lambda_{C\ max}$ 210 mμ, ε_C 8960, IR bands at 1760 and 1660 cm^{-1}). Pulchellin B had one hydroxyl group and what appeared to be an acetate (empirical formula, IR band at 1740 cm^{-1}); pulchellin C had two hydroxyl groups. The presence of a second double bond in both substances was indicated by IR absorption at 1645 cm^{-1} which established their bicyclic nature.

That pulchellin B was a monoacetate of pulchellin C became clear when it was found that both compounds furnished the same diacetate IV. The two hydroxyl group of pulchellin C were vicinal as evidenced by a positive periodate test. The presence of partial structure A in II and III was shown by the facile formation of a pyrazoline. Hydrogenation of IV with palladium–calcium carbonate saturated this

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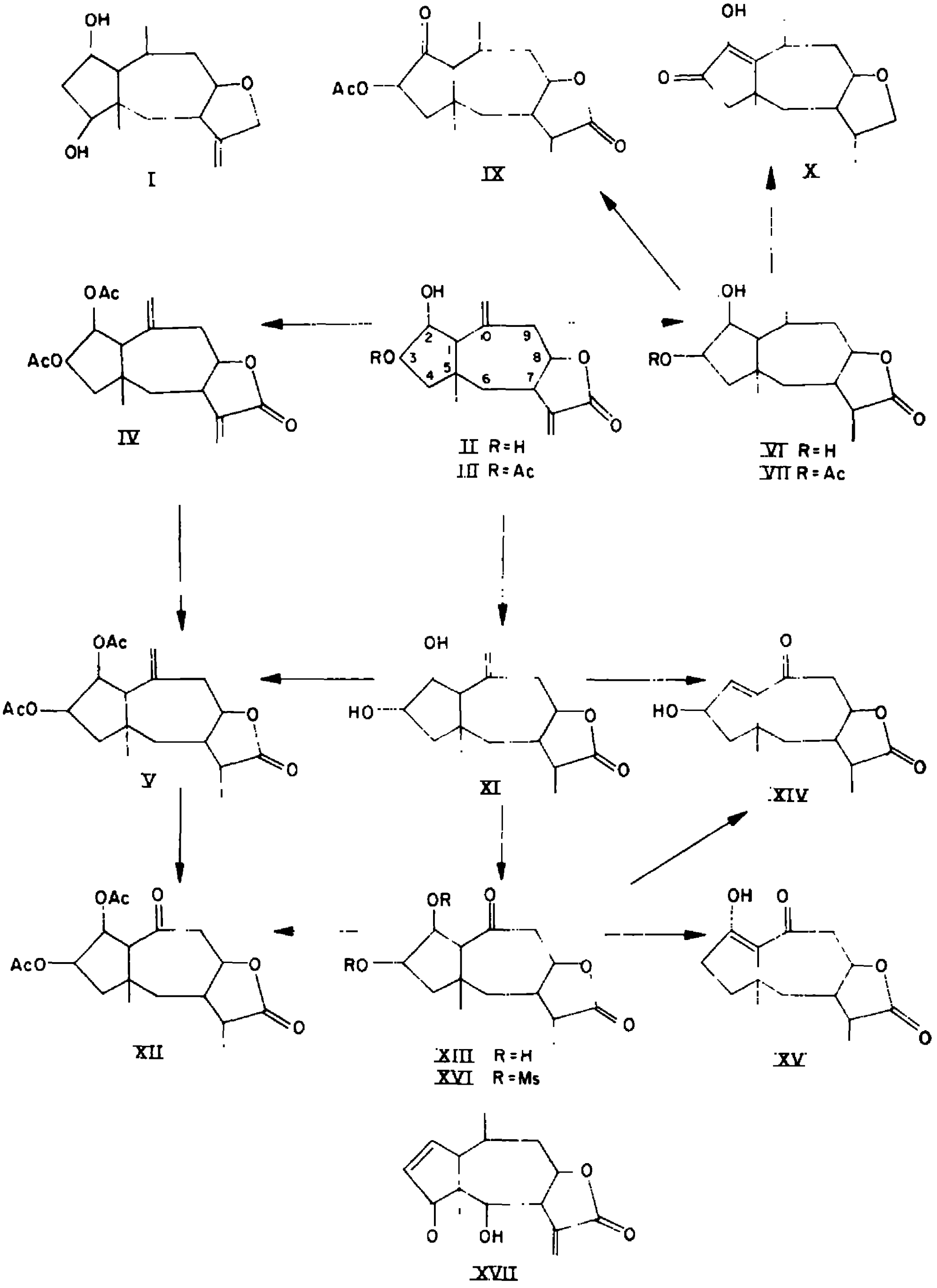
[1] Previous paper, W. Herz, K. Ueda and S. Inayama, *Tetrahedron* 19, 483 (1963). Supported in part by grants from the United States Public Health Service (RG-5814) and the National Science Foundation (NSF-G-14396).

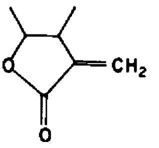
[2] We are greatly indebted to Dr. W. P. Stoutamire, Cranbrook Institute of Science, Bloomfield Hills, Mich., for valuable correspondence concerning these and related matters.

[3] Yields of pulchellin B, C and D by the trial and error procedure reported in the Experimental section were approximately 0·15, 0·35 and 0·007%. So far we have been unable to secure a second collection of the same cytotype, in order to establish more accurate percentages by a systematic fractionation of the extract.

[4] The eastern New Mexican races of *G. pulchella* Foug. tend to have pale rays and the pollen is often cream-coloured as opposed to the orange of other races. Chemical examination of other cytotypes of *G. pulchella* is obviously desirable and is now in progress.

functional grouping and led to the dihydroderivative V but left untouched the second apparently unconjugated double bond (no UV maximum, IR band at 1650 cm^{-1}), which could be reduced with platinum oxide. Hydrogenation of II, III and IV thus resulted in the three tetrahydroderivatives VI, VII and VIII.





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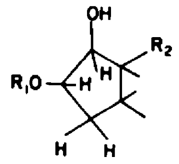
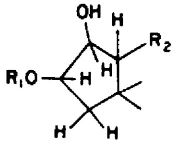


B R_1 = Ac
C R_1 = H



D R_1 = Ac

The following transformations permitted closer definition of the vicinal glycol system. Tetrahydropulchellin B(VII) was oxidized to an acetoxyketone IX whose infrared spectrum (bands at 1770, 1740 and 1725 cm^{-1}) suggested the presence of a cyclopentanone which had no α-methylene group (negative Zimmermann test). Pyrolysis of IX resulted in material whose UV (λ_{max} 221 mμ) and IR absorption (1700 and 1590 cm^{-1}) was in keeping with that of an unsubstituted α,β-unsaturated cyclopentanone, thus leading to partial structure B for III. Tetrahydropulchellin C(VI) was also oxidized, but to a diosphenol, whose UV (λ_{max} 278 mμ, 10090, in base λ_{max} 328 mμ) and IR spectrum (bands at 3500, 1770, 1665 and 1645 cm^{-1}) was wholly in accord with that of a substance X produced by oxidation of partial formula C.

The nature of the second double bond was elucidated in the following manner. Ozonolysis of dihydropulchellin C(XI) or its diacetate V resulted in liberation of formaldehyde and the formation of the apoketones XII and XIII both of which gave a positive Zimmerman test and therefore possessed free methylene groups next to the newly created carbonyl. The IR frequency (chloroform solution) of the latter (1745 cm^{-1} in XII superimposed on one of the acetate bands, 1725 cm^{-1} in XIII[6]) suggested initially that it was part of a five-membered ring, but the evidence to be discussed subsequently clearly ruled this out.

Table I lists NMR signals of various pulchellin B and C derivatives which confirm the conclusions reached so far and delineate further possibilities. To be noted initially are the characteristic doublets of the conjugated exocyclic methylene group present in III and IV which disappear on reduction to V or XI and are then replaced by a methyl doublet. III exhibits five additional low-field proton signals. Two of these must be associated with the unconjugated exocyclic methylene group, one being in clear evidence at 5·40 p.p.m., the second being superimposed on a signal associated with H_3.

In the spectrum of IV the more shielded vinyl proton of the second exocyclic methylene group can be discerned clearly at 4·70 p.p.m. The more deshielded one is

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[5] The isomeric diosphenol would have been expected to have a UV maximum at lower wave length than X. A satisfactory NMR spectrum of X which would have settled the matter could not be obtained because of solubility problems.

[6] A KBr pellet exhibited a more normal frequency at 1705 cm^{-1}.

TABLE 1. NMR PEAKS OF PULCHELLIN B AND PULCHELLIN C DERIVATIVES[a]

Compound	H_2	H_3	H_8	H_{13}	H_{14}	C_5–Me	C_{10}–Me	C_{11}·Me	Misc.
III	4·0dbr(10)	4·85m^b	4·5c	6·18d(1) 5·62d(1)	5·40br 4·80br^b	0·91	—		2·08^c
IV	5m^b	5m^b	4·48td(4)	6·12d(1) 5·60d(1)	5^b 4·70	0·92	—		2·11^c 2·00^c
V	4·85m^b	4·85m^b	4·34c	—	4·8^b 4·62	0·90		1·19d(7)	2·04^c 1·92^c 2·75q(6)^d
VII	3·68dd(11,6)	5·00td(11,4)	4·45td	—	—	1·10	0·94d(8)	1·22d(8)	2·08^c 2·78q(6)^d
VIII	4·80dd(11,6)	5·08td(11,4)	4·27c	—	—	1·10	0·89d(7)	1·18d(7)	1·98^c 1·92^c 2·68q(6)^d
IX	—	5·61dd(13,6)	4·48m	—	—	1·32	1·22d(8)	1·22d(8)	
XII	5·11m^b	5·11m^b	4·30c	—	—	0·90	—	1·16d(7)	2·08^c 1·98^c 2·72q(6)^d

[a] Spectra determined on a Varian A-60 spectrometer in deuteriochloroform solution. Values given in p.p.m. relative to tetramethylsilane as internal standard. All signals in first five columns correspond to one proton, all signals in last four to three unless otherwise specified. Singlets are unmarked, multiplets are described as follows: d doublet, dd doublet of doublets, t triplet, q quartet, br somewhat broadened singlet or ill-defined doublet, m multiplet of uncertain multiplicity, c complex signal whose center is given. Numbers in parentheses denote coupling constants in c.p.s. [b] Superimposed on other signal. [c] Acetate. [d] H_{11}.

obscured by two sets of signals near 5 p.p.m. One of these which has moved downfield from 4 to 5 p.p.m. on acetylation, is due to H_2, the other due to H_3. Hydrogen on carbon carrying the lactonic ether oxygen, in III merely a complex band near 4·5 p.p.m., is clearly seen to be spin-coupled to three protons in IV.

Comparison of the NMR spectrum of V with that of the apoketone XII indicates the correctness of these assignments. V exhibits low-field protons three of which—one vinyl proton and two hydrogens on carbon carrying acetoxyl groups—are superimposed on each other near 4·85 p.p.m. The fourth signal, at 4·62 p.p.m., must be ascribed to the second vinyl proton, the fifth to the lactone hydrogen. In the apoketone XII, the number of low-field protons has dropped to three, two—those at C_2 and C_3—being superimposed. Mention must also be made of the persistent signal of a tertiary methyl group near 1 p.p.m. which has to be accommodated in a plausible structural formula.

In the spectrum of tetrahydropulchellin B(VII), which has two methyl doublets and one methyl singlet, there is found a doublet of triplets at 5 p.p.m. which must be associated with hydrogen on carbon carrying the acetoxy group (H_3) since the lactonic hydrogen reveals its presence at the usual position near 4·5 p.p.m. The doublet of doublets at 3·68 p.p.m. may therefore be assigned to hydrogen on carbon carrying the free hydroxyl group—a conclusion which is fully confirmed by the downfield shift of this signal to 4·8 p.p.m. on acetylation to VIII and its disappearance on oxidation of VII to IX. Simultaneously, the multiplicity of H_3 changes in accordance with partial structure B which, since H_2 is clearly spin-coupled to two protons, may be expanded to D[7]. Since the pulchellins are bicyclic, the second ring must be fused to C_5 of the cyclopentane nucleus or incorporated in some way in R_2.

A decision between these possibilities could be reached quite simply. An attempt to acetylate the apoketone XIII led to dehydration and the formation of an α,β-unsaturated ketone XIV which was not a cyclopentenone (λ_{max} 235 mμ, ε 8075, IR bands at 3500, 1770, 1685 and 1650 cm^{-1}). This indicated that the second ring was six-membered or larger and that the carbonyl group—and therefore the exocyclic methylene group from which it derives—was β to one of the hydroxyls on the five-membered ring.

Confirmation for this relationship of functional groups was provided by a formic acid-catalyzed pinacol rearrangement of XIII which furnished the enolic β-diketone XV. This substance, λ_{max} 276 mμ (9500), λ_{max} base 278 and 320 mμ (ε 8270 and 2850), was also obtained from the dimesylate XVI by treatment with sodium acetate.

Now it will be remembered that the unconjugated methylene group adjoins —CH_2—. Any attempt to incorporate the remaining features—partial structure A whose ether oxygen is secondary and adjacent to carbon atoms carrying a total of three protons, as well as a tertiary methyl group reduces uniquely to formulae II and III if it is assumed that the potential isopropyl side chain is attached to C_7 as is required by the usual biogenetic schemes and found in all other sesquiterpene lactones isolated from *Helenium* and *Gaillardia* species.

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[7] In the absence of other information we hesitate to deduce the relative stereochemistry of the five-membered ring from the apparent coupling constants $J_{H_1H_2} \sim 6$, $J_{H_2H_3} \sim 11$, $J_{H_3H_{4a}} \sim 11$, $J_{H_3H_{4b}} \sim$ 4–5, although it would appear that H_2 and H_3 are *cis*. The element of uncertainty is due to $J_{H_1H_2}$ being intermediate between $J_{H_3H_{4a}}$ (*cis*) and $J_{H_3H_{4b}}$ (*trans*), although it is closer to the latter.

Support for this assumption was provided by lithium aluminum hydride reduction followed by dehydrogenation of a mixture of pulchellin B and C. Although yields were very low, the formation of 1,4,7-tri- and 4,7-dialkyl-substituted azulenes was demonstrated spectroscopically thus establishing the carbon skeleton.

Insufficient material was available for structure determination of pulchellin D, $C_{17}H_{24}O_5$, m.p. 182–185°. The lack of ultraviolet absorption and the infrared spectrum-bands at 3600, 1763 (shoulder at 1750) and 1650 cm^{-1} suggested that pulchellin D was dihydropulchellin B or an isomer thereof. It should be possible to test this by partial reduction of pulchellin B when additional material becomes available.

Extraction of *Gaillardia megapotamica* (Spreng.) Baker, the sole South American representative of the genus *Gaillarda* (section *Eugaillardia*), and *Gaillardia multiceps* Greene, a species of limited distribution in Arizona and New Mexico[8] (section *Agassizia*), furnished helenalin[9] (XVII) in 1·1 and 1·0% yield. Helenalin has been isolated previously from a large number of *Helenium* species[10] and from *Balduina angustifolia* Nutt.[11] Its occurrence in *Gaillardia* species further corroborates the very close relationship which has been postulated to exist between the two genera.

EXPERIMENTAL[12]

Isolation of Pulchellin B, C *and* D. *Gaillardia pulchella* Foug., wt. 4·2 lbs. (Rock No. 1102) was collected by Dr. H. F. L. Rock on July 3, 1960 along route 66, at Tucumcari, Quay Co., New Mexico. Extraction with chloroform by the usual procedure furnished a gum which was chromatographed over alumina (Alcoa, F-20) in the usual way to purify what was expected to be pulchellin. Seven 100 ml fractions of benzene–chloroform (1:3), six 100 ml fractions of chloroform–methanol (9:1) and one 300 ml fraction of methanol were collected.

Fractions 2 and 3 (1·2 g solid material) were combined and recrystallized 3 times from acetone–pentane, m.p. 210–213° (pulchellin B). Fractions 4 and 5 (wt. 1·5 g) were combined and recrystallized twice from acetone–pentane, m.p. 205–210° (slightly impure pulchellin B). Fractions 6–8 on recrystallization furnished a small amount of pulchellin B.

Fraction 10, wt. 3·2 g, was recrystallized from acetone–pentane, but the m.p. remained unsharp (193–200°) and the IR spectrum and thin layer chromatogram indicated the presence of pulchellin B and pulchellin C. Rechromatography over 500 g acid-washed alumina and elution with 250 ml solvent portions of increasing polarity furnished the following: fractions 6–8 (benzene), 0·02 g, m.p. 205–215° (impure pulchellin B), fractions 15–16 (chloroform–acetone 1:1), 0·08 g, m.p. 215–218° after recrystallization from acetone–pentane (analytically pure pulchellin B), fractions 30–33 (acetone–methanol 2:1), 3·02 g, m.p. 186–199°, raised to 199–202° after recrystallization from acetone–pentane (analytically pure pulchellin C).

Fractions 9 and 11 of the original chromatogram (wt. 5·2 g) were combined, dissolved in benzene and chromatographed over 500 g acid-washed alumina in the same way. Fractions 6–8 (benzene) gave 0·035 g pulchellin B, m.p. 213–217°, fractions 11 and 12 (benzene–chloroform, 1:1) gave 0·11 g pulchellin B, m.p. 213–218°, fractions 15 and 16 (chloroform) gave pulchellin D, wt. 0·12 g, m.p. 178–182°, raised to 182–185° after recrystallization from acetone–pentane, depression on admixture of pulchellin, pulchellin B and pulchellin C, fractions 17–18 (chloroform–acetone 1:1), 0·04 g

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[8] S. F. Biddulph, *Research Studies Washington State Coll.* **12**, No. 4, p. 195 (1944).

[9] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *J. Amer. Chem. Soc.* **85**, 19 (1963).

[10] W. Herz, *J. Org. Chem.* **27**, 4043 (1962).

[11] W. Herz and R. B. Mitra, *J. Amer. Chem. Soc.* **80**, 4876 (1958).

[12] M.ps and b.ps are uncorrected. Analysis by Dr. F. Pascher, Bonn, Germany. IR Spectra were run in chloroform and rotations in 95% ethanol solution unless otherwise specified, UV spectra in 95% ethanol. NMR spectra were run on an A-60 instrument in deuteriochloroform solution, tetramethylsilane serving as internal standard. The NMR spectrometer was purchased with the aid of a grant from the National Science Foundation.

slightly impure pulchellin B, fractions 27–28 (acetone–methanol 3:1) 3·1 g pulchellin C, m.p. 198–201°.

Rechromatography of the mother liquors of fractions 2–5 of the original chromatogram furnished additional small amounts of pulchellin B and pulchellin C.

Analytically pure pulchellin B melted at 215–218°, $[\alpha]_D^{27}$ +92·7° (95% ethanol, *c*, 1·54), λ_{max} 210 and 325 mμ (ε 8830 and 53), IR bands ($CHCl_3$) 3560 (—OH), 1760 (γ-lactone), 1740 (acetate), 1660 and 1645 cm^{-1} (double bonds), (nujol) 3555, 1755, 1740, 1665 and 1645 cm^{-1}. (Found: C, 66·64; H, 7·13; O, 26·49; Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24; O, 26·11%.)

Analytically pure pulchellin C melted at 199–202°, $[\alpha]_D^{27}$ +125° (95% ethanol, *c*, 1·55), λ_{max} 210 mμ (ε 8960), IR bands ($CHCl_3$) 3700 and 3600 (—OH), 1760 (γ-lactone), 1660 and 1645 cm^{-1} (double bonds), (nujol) 3800, 3550, 1750, 1670 and 1650 cm^{-1}. (Found: C, 67·83; H, 7·70; O, 24·70; Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

Analytically pure pulchellin D melted at 182–185°, no UV absorption, IR bands at 3600 (—OH), 1763 (γ-lactone, shoulder at 1750) and 1650 cm^{-1} (double bond). Thin-layer chromatography clearly distinguished this substance from pulchellin, pulchellin B and pulchellin C. (Found: C, 65·76; H, 7·56; O, 26·29; Calc. for $C_{17}H_{24}O_5$: C, 66·21; H, 7·85; O, 25·94%.)

Dehydrogenation. Material from various mother liquors containing only pulchellin B and pulchellin C (thin layer chromatogram), wt. 0·9 g, was reduced with $LiAlH_4$ (Soxhlet technique) and worked up in the usual fashion. The resultant gum, wt. 0·5 g, was suspended in a petroleum fraction, b.p. 300°, and dehydrogenated with 1 g of 10% Pd–C in a N_2 atmosphere. The mixture was diluted with hexane and extracted with 90% phosphoric acid. The acid extract was washed with hexane, diluted with water, extracted with hexane and the organic layer chromatographed over 10 g alumina. Several fractions were obtained. A faster-moving zone was blue, visible spectrum coincident with that of chamazulene, but the amount of material available was insufficient for the preparation of a sharply melting trinitrobenzene complex. Paper[13] and thin-layer chromatography indicated the presence of impurities but the main spot had an R_f value coincident with the R_f value of chamazulene. A more polar zone was blue-violet, again insufficient for the preparation of derivatives but identified as probably mainly linderazulene by spectrum and chromatography. (R_f value of main spot coincident with that of linderazulene.) A third zone had a visible spectrum similar to 4,7-dialkylazulenes[14], but the chromatogram indicated several spots.

Diacetylpulchellin C (IV). A solution of 0·2 g V, 2 ml acetic anhydride and 1 ml pyridine was allowed to stand for several hours, diluted with ice water and filtered. The crude product, wt. 0·24 g m.p. 184–187°, was recrystallized from aqueous methanol, m.p. 190–193°, $[\alpha]_D^{28}$ +94·5° (*c*, 1·0), IR bands at 1753 (acetates and lactone) and 1653 (broad, double bonds). It gave a negative periodic acid test. The same compound, m.p. 189–191°, was obtained from pulchellin B, m.p. undepressed, IR spectra superimposable. (Found: C, 65·68; H, 7·29; O, 27·13; Calc. for $C_{19}H_{24}O_6$: C, 65·50; H, 6·94; O, 27·56%.)

Pyrazolines. A solution of 0·05 g pulchellin B in 3 ml methanol was mixed with excess ethereal diazomethane solution and allowed to stand at 5° for 5 days. Filtration and evaporation at room temp followed by recrystallization from aqueous methanol furnished needle-like prisms, m.p. 164–165° (dec.). (Found: C, 61·95; H, 7·14; N, 8·04; Calc. for $C_{18}H_{24}O_5N_2$: C, 62·05; H, 6·94; N, 8.04%.)

The pyrazoline of pulchellin C, silky needles from aqueous methanol, melted at 165–167° (dec.). (Found: C, 59·68; H, 7·88; N, 8·48; Calc. for $C_{16}H_{22}O_4N_2$: C, 59·24; H, 7·46; N, 8·64%.)

Dihydropulchellin C (XI). A solution of 0·3 g pulchellin C in 30 ml ethanol was hydrogenated with 0·03 g Pd–$CaCo_3$ catalyst in a Parr hydrogenator at 30 lbs hydrogen pressure (hydrogenation at atm. press. with PtO_2 resulted in less pure product). The solvent was removed and the product, wt. 0·3 g, m.p. 205–208°, was recrystallized from aqueous methanol.

The colorless prisms melted at 207–210°, $[\alpha]_D^{28}$ +5·32° (*c*, 1·90), no UV absorption, IR bonds (nujol) at 3600, 3400, 1765 and 1650 cm^{-1}. (Found: C, 63·40; H, 8·55; O, 28·30; Calc. for $C_{15}H_{22}O_4 \cdot H_2O$: C, 63·36; H, 8·51; O, 28·14%.)

Diacetyldihydropulchellin C (V). A solution of 0·075 g IV in 10 ml methanol was hydrogenated with 0·02 g Pd–$CaCo_3$ at 30 lbs press. Filtration, evaporation and recrystallization of the residue from aqueous methanol afforded 0·3 g V, m.p. 238–239°, $[\alpha]_D^{24}$ −103° (*c*, 1·04, methanol), no UV

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[13] V. Sýkora and K. Vokáč, *Coll. Czechoslov Chem. Commum.* **25**, 1702–1704 (1960).

[14] H. Pommer, *Liebig's Ann.* **579**, 47 (1952).

absorption, IR bands at 1775, 1740 (double strength) and 1650 cm^{-1}. This substance was also prepared in 90% yield by acetylation of dihydropulchellin. (Found: C, 64·73; H, 7·46; O, 27·67; Calc. for $C_{19}H_{26}O_6$: C, 65·12; H, 7·48; O, 27·40%.)

Tetrahydropulchellin C (VI). A solution of 0·1 g pulchellin C in 25 ml methanol was hydrogenated in the presence of 0·01 g PtO_2 at room temp. and atm. press. Hydrogen uptake ceased after absorption of 19·8 ml (calc. for 2 moles 18·8 ml). The usual work-up afforded colorless crystals, wt. 0·1 g, m.p. 97–109°, which were recrystallized repeatedly from acetone–pentane and then melted at 118–120°, yield 0·1 g, $[\alpha]_D^{24}$ $-25·2°$ (*c*, 1·87), no UV maximum, IR bands at 3700 and 1775 cm^{-1}. Better yields were realized subsequently by carrying out the hydrogenation in a Parr hydrogenator. This compound was also obtained by further hydrogenation of XI (PtO_2, 30 lbs press). (Found: C, 62·91; H, 9·15; O, 27·94; Calc. for $C_{15}H_{24}O_4 \cdot H_2O$: C, 62·58; H, 9·43; O, 28·04%.)

Diacetyltetrahydropulchellin C (VIII). A solution of 0·1 g diacetylpulchellin C in 25 ml methanol was hydrogenated as described in the preceding paragraph, hydrogen uptake found 13·3 ml, calc. 12·8 ml. The product was recrystallized from acetone–pentane, yield 0·06 g, m.p. 227–230°, $[\alpha]_D^{23}$ $-66·8°$ (1·00, methanol), no UV maximum, IR bands at 1775 and 1735 cm^{-1} (double strength). This substance was also prepared by hydrogenation of V and acetylation of VI. (Found: C, 64·60; H, 8·15; O, 27·18; Calc. for $C_{19}H_{28}O_6$: C, 64·75; H, 8·01; O, 27·27%.)

Tetrahydropulchellin B (VII). A solution of 0·1 g pulchellin B in 20 ml ethanol was hydrogenated with 0·01 g PtO_2 at 30 lbs press. The usual work-up furnished 0·1 g solid which was recrystallized from aqueous methanol, m.p. 200–204°, $[\alpha]_D^{24}$ $+40·2°$ (*c*, 1·2), IR bands at 3600, 1770 and 1740 cm^{-1}. Acetylation (acetic anhydride–pyridine) gave VIII. (Found: C, 65·49; H, 8·71; O, 25·90; Calc. for $C_{17}H_{26}O_5$: C, 65·78; H, 8·44; O, 25·78%.)

Dehydrotetrahydropulchellin B (IX). To 0·1 g VII in 1 ml acetic acid was added dropwise with stirring a solution of 0·06 g chromic oxide in 5 ml acetic acid. Stirring was continued for 24 hr, excess oxidizing agent destroyed with methanol and the solvent removed *in vacuo* at room temp. The residue was diluted with water, the crystalline product collected, washed (yield 0·05 g) and recrystallized from aqueous methanol, m.p. 201–204°, depression on admixture of VII, negative Zimmermann test, $[\alpha]_D^{20}$ $+25·4°$ (*c*, 1·26), IR bands at 1770, 1745 and 1725 cm^{-1}. (Found: C, 66·25; H, 7·68; O, 26·11; Calc. for $C_{17}H_{26}O_5$: C, 66·21; H, 7·85; O, 25·94%.)

The ketoacetate, wt. 0·1 g, was heated in an oil bath at 280–300° for 10 min, the brown product dissolved in methanol, treated with charcoal and the solution evaporated at red. press. The solid residue exhibited λ_{max} 221 mμ, IR bands at 1775, 1700 and 1590 cm^{-1}.

Bis-dehydrotetrahydropulchellin C (X). To a solution of 0·5 g VI in 20 ml acetic acid was added dropwise with stirring a solution of 0·5 g chromic oxide in 30 ml acetic acid. Stirring was continued overnight, methanol was added, the mixture evaporated *in vacuo*, the residue diluted with water and thoroughly extracted with ether and methylene chloride. The organic extracts were combined, washed, dried and concentrated to small volume whereupon prisms deposited, m.p. 235–238°, yield 0·04 g, $[\alpha]_D^{20}$ $+33·8°$ (*c*, 1·51, acetone), λ_{max} 278 mμ (ε 10090), λ_{max} on addition of base 328 mμ (ε 2100), IR bands (KBr pellet) 3500, 1770, 1665 and 1645 cm^{-1}, positive ferric chloride test. Attempts to oxidize VI with chromic oxide–pyridine resulted in recovery of starting material. (Found: C, 68·25; H, 7·74; O, 24·35; Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21%.)

Diacetylapodihydropulchellone C (XII). A solution of 0·2 g V in 50 ml methanol was ozonized at $-60°$ for 2 hr and then steam-distilled into a refrigerated solution of dimedone in methanol. Concentration of the dimedone solution resulted in isolation of 0·02 g formaldehyde derivative. The material remaining in the flask was concentrated *in vacuo*; the residue, wt. 0·1 g, m.p. 202–205°, was recrystallized from aqueous methanol. The needle-like prisms of XII melted at 222–224°, $[\alpha]_D^{23}$ $-61·7°$ (*c*, 3·20), no UV absorption, IR bands at 1775, 1750 and 1745 cm^{-1}, (nujol) 1765, 1750 and 1735 cm^{-1}, positive Zimmermann test. (Found: C, 61·48; H, 6·84; O, 31·87; Calc. for $C_{18}H_{24}O_7$: C, 61·35; H, 6·86; O, 31·78%.)

Apodihydropulchellone C (XIII). A solution of 0·2 g dihydropulchellin C in 50 ml methanol was ozonized at $-60°$ for 2 hr, and the solution hydrogenated with 0·02 g Pd–$CaCO_3$ in a Parr hydrogenator. Filtration followed by evaporation gave solid material (XIII) wt. 0·18 g, m.p. 209–215°, which was recrystallized from methanol and then melted at 218–221°, $[\alpha]_D^{23}$ $+7·90$ (*c*, 1·00), no UV absorption, IR bands at 3600, 3200, 1770 and 1725 cm^{-1} (KBr pellet), 3600, 1770 and 1705 cm^{-1}, positive Zimmermann test. (Found: C, 62·91; H, 7·87; O, 29·59; Calc. for $C_{14}H_{20}O_5$: C, 62·67; H, 7·51; O, 29·82%.)

Dehydration of XXIII. A solution of 0·1 g XIII in 2 ml acetic anhydride and 1 ml pyridine was allowed to stand for 2 hr and poured into ice water. The crude diacetate, wt. 0·1 g, was recrystallized from aqueous methanol. The product, m.p. 222–224°, did not depress the m.p. of authentic XII.

In an attempt to repeat this preparation with 0·03 g XIII, 2 ml acetic anhydride and 1 ml pyridine, the mixture was decomposed with tepid instead of ice water. Concentration of the mixture furnished solid material which was recrystallized from aqueous methanol. The colorless needles (XIV) melted at 230°, yield 0·015 g, λ_{max} 235 mμ (ε 8075), IR bands (KBr pellet) at 3500, 1770, 1685 and 1650 cm^{-1}. (Found: C, 66·17; H, 7·08; O, 26·82; Calc. for $C_{14}H_{18}O_4\cdot\frac{1}{4}H_2O$: C, 65·99; H, 7·32; O, 26·71%.)

Pinacol rearrangement of XIII. A solution of 0·2 g XIII in 2 ml formic acid was refluxed for 4 hr, evaporated at red. press. and the residue diluted with water. Extraction with chloroform, washing and drying gave a gum which was recrystallized from aqueous methanol. The pale yellow prisms (XV) melted at 127–130°, $[\alpha]_D^{21}$ +89·4° (*c*, 0·95, acetone), λ_{max} 276 mμ (ε 9500), λ_{max} on addition of base 278 and 320 mμ (ε 8270 and 2850), IR bands (KBr pellet) 3500, 1785, 1660 and 1635 cm^{-1}, positive ferric chloride test. The material was a solvate containing water or methanol. (Found: C, 65·39; H, 7·30; O, 27·91; Calc. for $C_{14}H_8O_4\cdot\frac{1}{2}H_2O$: C, 64·84; H, 7·39; O, 27·77; Calc. for $C_{14}H_{18}O_4\cdot\frac{1}{2}CH_3OH$: C, 65·40; H, 7·50; O, 27·04%.)

XV was also prepared by the following sequence.

A solution of 0·2 g XIII in 1 ml pyridine was mixed with 0·3 g methane–sulfonyl chloride, kept in the refrigerator overnight and poured into ice water. The crude mesylate XVI was filtered, washed and dried, m.p. 237–240°, wt. 0·2 g, IR bands at 1775 and 1705 cm^{-1}. It was refluxed with 0·3 g sodium acetate in ethanol for 20 hr and evaporated *in vacuo*. The residue was extracted with chloroform, the organic extract washed, dried and evaporated and the oily residue allowed to stand with methanol–hydrochloric acid overnight. Removal of solvents followed by recrystallization from aqueous methanol furnished pale yellow crystals (positive ferric chloride test), m.p. 125–128°, which were identical with XV by direct comparison.

Extraction of Gaillardia multiceps *Greene*. Dried leaves, flowerheads and small stems of *G. multiceps* supplied by Dr. W. P. Stoutamire and described as being derived from seed collected at localities in West Texas, Southern New Mexico and Arizona (W.P.S. accession numbers 2522, 2525, 2529, 2546, 2548 and 2549), wt. 640 g, was extracted with chloroform and worked up in the usual way, yield 11·5 g crude gum. This was dissolved in 40 ml chloroform, mixed with 20 ml benzene and chromatographed over 150 g Alcoa F-20 alumina. Six 100 ml fractions of benzene–chloroform (1:1) eluted 5·35 g crystalline material, chloroform eluted an additional 1·9 g solid. Recrystallization from acetone–pentane and benzene–pentane furnished 6·45 g material, m.p. 162–164°, identical in all respects with authentic helenalin.

Extraction of Gaillardia megapotamica (*Spreng.*) *Baker*. Dried leaves, flowerheads and small stems supplied by Dr. Stoutamire and described as being grown from seed collected at Mar Chiquita, Cordoba, Argentina (W.P.S. accession number 2658), wt. 150 g, were extracted with chloroform and worked up as usual, yield of crude gum 2·2 g. This was dissolved in 15 ml hot benzene. The solution was allowed to cool and chromatographed over 30 g Alcoa F-20 alumina. Benzene eluted 0·4 g crystalline material, chloroform eluted 1·7 g solid. Recrystallization of both fractions from acetone-hexane furnished 1·65 g helenalin, m.p. 159–163°, identical in all respects (IR spectrum, thin layer chromatogram) with authentic material.

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