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CONSTITUENTS OF *GAILLARDIA* SPECIES—I

THE STRUCTURE OF PULCHELLIN[1]

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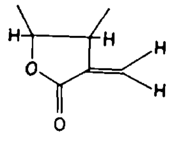
Abstract—The structure of pulchellin, a sesquiterpene lactone from *Gaillardia pulchella* Foug., is shown to be Ia.

The genus *Helenium* elaborates a plethora of interesting sesquiterpene lactones[2] whose structures and transformations have been studied intensively in the past few years. Its closest relative in the family *Compositae* is the genus *Gaillardia.* Its distribution, like that of *Helenium* species, is limited to the Western Hemisphere.

Because of our interest in sesquiterpene lactones and in the phylogenetic relationships existing in this group of plants, we have undertaken a chemical examination of *Gaillardia* species.[3,4] In this paper we report on the isolation and structure of pulchellin, a new sesquiterpene lactone from *Gaillardia pulchella* Foug. Subsequent papers will deal with the constituents of the other members of this genus.

Extraction of *Gaillardia pulchella* collected in Florida or North Carolina[5] gave in approximately 0·17% yield a substance $C_{15}H_{22}O_4$, m.p. 165–168°, $(\alpha)_D^{26}$ −36·2°, which appeared to be new and which we have named pulchellin. Pulchellin had two hydroxyl groups (I.R. band at 3570 cm^{-1}, formation of a diacetate) and one double bond (I.R. band at 1660 cm^{-1}, hydrogenation). The remaining two oxygen atoms were accounted for by assuming the presence of a γ-lactone group (band at 1760 cm^{-1}) which was conjugated with the double bond (λ_{max} 209 mμ, ϵ 9400). Hence pulchellin was assumed to be bicyclic.

The nature of the double bond was inferred from the U.V. maximum and from



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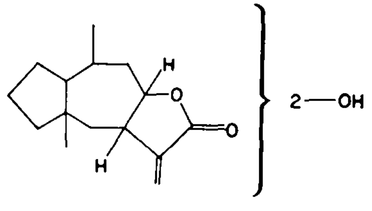

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[1] Supported in part by grants from the United States Public Health Service (RG-5814) and the National Science Foundation (NSF-G-14396).

[2] For a summary, see W. Herz, *J. Org. Chem.* **27**, 4043 (1962).

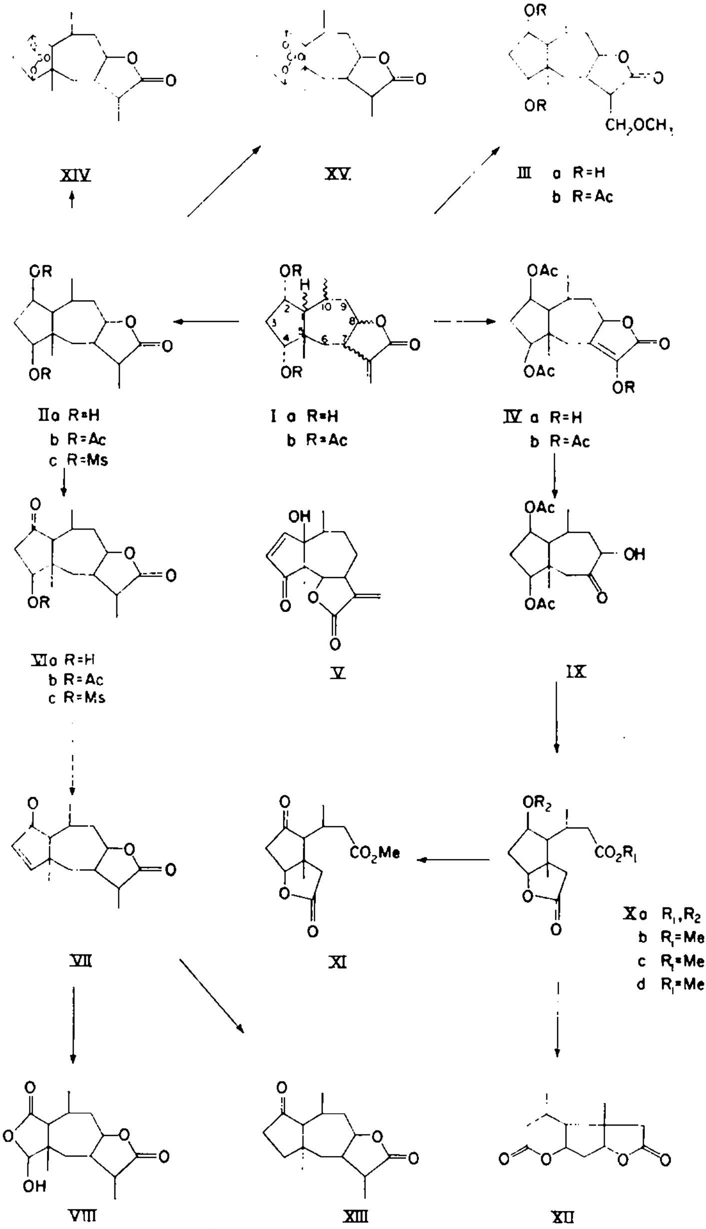
[3] The most recent revision of the genus *Gaillardia* is that of S. F. Biddulph, *Res. Studies, Wash. State Coll.* **12**, No. 4, 195 (1944).

[4] The relationship of anthocyanin content to *Gaillardia* taxonomy has been studied by W. P. Stoutamire, *Papers Mich. Acad. Sci. Arts and Lett.* **45**, 35 (1960).

[5] *G. pulchella* Foug., is widespread throughout the Southwestern United States. It is also found in two isolated pockets in the Southeast—in North-eastern Florida and Eastern North Carolina—and is frequently grown as an ornament elsewhere. The coastal races of the Southeast are closely allied to the coastal races of the Southwest but differ somewhat more widely from the numerous high plains races.[6] This difference seems to be reflected in the sesquiterpene lactone content (unpublished work by S. Inayama).

[6] Private communication from Dr. W. P. Stoutamire.

ozonolysis (liberation of formaldehyde). This pointed to the presence of partial structure A (the necessity for one hydrogen atom each at the β and γ position of A will be apparent from the subsequent discussion), as did a comparison of the N.M.R. spectra of pulchellin (Ia) and pulchellin diacetate (Ib) with those of the dihydro derivatives IIa and IIb. Ia and Ib exhibited the characteristic low field doublets at 6·15 and 5·47 p.p.m., J = 3 c.p.s., of the exocyclic methylene group conjugated with a lactone carbonyl.[7] These were missing in the spectra of IIa and IIb, which, however, exhibited an additional methyl doublet (Table 1).



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[7] W. Herz, H. Watanabe, M. Miyazaki, and Y. Kishida, *J. Amer. Chem. Soc.* **84**, 2601 (1962).

TABLE 1. N.M.R. PEAKS OF PULCHELLIN DERIVATIVES[a]

Compound	H_2	H_3	H_4	H_8	H_{14}	C_5—Me	C_{10}—Me	C_{11}—Me	Misc.
Ia	4·1c		3·65d (4)	4·2c	6·15d (3) 5·47d (3)	0·90	1·37d (5)		
Ib	5·03[b]		4·78d (5)	4·00c	6·15d (3) 5·40d (3)	0·98	1·02d (7)		2·01[c] 2·06[c]
IIa	4·0c		3·59d (5)	4·1c		0·83	1·21d (5)	1·21d (5)	
IIb	5·0e		4·75d (5)	4·00c		0·98	1·04d (6)	1·21d (6)	2·03[c] 2·08[c]
IIIb	5·02c		4·75d (6)	4·02t (7)		0·97	1·03d (6·5)	3·63d (2)[d]	2·03[c], 3·30[e] 2·10[c]
IVa	5·05c		4·82d (4)	5·20c	2·83br[f] 2·70br[f]	1·02	1·03d (4)		2·09[c] 2·14[c]
IVb	5·08c		4·76d (4)	5·15c	2·75br[f] 2·65br[f]	1·02	1·05d (4)		2·03[c] 2·14[c]
VII	7·18d (6)	6·15d (6)		3·78c		1·31	1·06d (8)	1·44d (6)	
Xb	4·4c		4·46dd (3·5, 3)			1·30	1·19d (5)		3·79[f]
Xc	5·12td (8, 3)		4·47dd (3, 2)			1·18	0·98d (5·5)		3·82[f]
XII	4·80e		4·43dd (4, 3)			1·27	1·20d (6)		

[a] Spectra were run by Mr. Fred Boerwinkle in deuteriochloroform solution on a Varian HR-60 spectrometer. Frequencies were determined by the side band technique. Funds for the purchase of the spectrometer were supplied by the Institute of Molecular Biophysics of the Florida State University. Values are given in p.p.m. relative to tetramethylsilane as internal standard. Numbers in parentheses denote coupling constants in c.p.s. All signals in first five columns correspond to one proton, all signals in last four to three. Singlets are unmarked; multiplets are described as follows: d doublet, dd doublet of doublets, t triplet, br somewhat broadened singlet or ill-defined doublet, c complex signal whose center is given.

[b] X component of AB_2X system. [c] Acetate. [d] Methylene (2 protons). [e] Methoxyl. [f] H_6 proton.

Furthermore, methanolic potassium hydroxide converted pulchellin to the methoxy derivative IIa[8] in the manner described in the interim[9] for other sesquiterpene lactones of this type. Lastly, ozonolysis of Ib in methanol at −70° resulted in the formation of the enolic α-ketobutyrolactone diacetylnorpulchellone (IVa), whose properties (positive ferric chloride test, I.R. and U.V. spectrum, formation of an enol acetate IVb) completely duplicated those of an analogous compound obtained by low temperature ozonolysis of parthenin (V).[7]

Lithium aluminum hydride reduction of pulchellin and dihydropulchellin followed by dehydrogenation afforded only traces of azulenes and no benzenoid or naphthalenic hydrocarbons. This excluded formulae based on the guaiane or eudesmane skeleton. Since pulchellin was bicyclic and had one tertiary and one secondary methyl group (Table 1), it was logical to assume, as a working hypothesis, that its structure was based on the "abnormal" carbon skeleton (partial structure B) deduced previously for the sesquiterpene lactones of *Helenium* species.[10–12] Lactone ring closure to C_8 would be required if this supposition were correct in view of the complexity of the N.M.R. signal near 4 p.p.m. (Table I), ascribable to the lactone ether proton.[10] Additional signals in this region, one a doublet, J = 4 c.p.s., the second more complex, both of which moved downfield on acetylation, suggested that the two hydroxyl groups were secondary, a deduction which was supported by the facile preparation of a dimesylate.

After considerable experimentation conditions were found for the selective oxidation of one of the hydroxyl groups of dihydropulchellin (IIa). The product, dehydrodihydropulchellin (VIa), was a cyclopentanone (infra-red bands at 3500, 1775, and 1750 cm^{-1}), thus providing support for the working hypothesis. Acetylation and mesylation proceeded smoothly and yielded VIb and VIc.

The mesylate VIc on treatment with lutidine or sodium acetate, or on prolonged exposure to drying agents at elevated temperatures, afforded an α,β-unsaturated ketone VII, λ_{max} 220 mμ (ε10300) which was clearly a cyclopentenone derivative (infra-red bands at 1770—lactone—and 1705 and 1595 cm^{-1}—cyclopentenone[13]). Hence, unless a rearrangement accompanies the elimination reaction, VIa is a β-hydroxycyclopentanone, and pulchellin a 1,3-diol, in which both hydroxyl groups are secondary and at least one of the hydroxyl groups is located in a five-membered ring.

VII could also be prepared directly from VIa under carefully defined conditions, but the yields were poor. Its N.M.R. spectrum had two doublets (intensity one proton each) at 7·18 and 6·15 p.p.m. (J = 6 e.p.s.) which are characteristic of unsubstituted α,β-unsaturated cyclopentenones.[7] The lack of further splitting indicated that the γ-carbon was completely substituted. Other signals included two methyl doublets, one of which resulted from the reduction of partial structure A), the usual

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[8] Chromatography over basic alumina of the mother liquors from the recrystallization of pulchellin also afforded III in the last, methanol eluate due to reaction on the column.

[9] G. H. Kulkarni, A. Paul, A. S. Rao, G. R. Kelkar, and S. C. Bhattacharya, *Tetrahedron* **1**, 178 (1961).

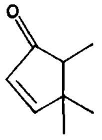
[10] W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman, and N. Viswanathan, *J. Amer. Chem. Sov.* **84**, 3857 (1962).

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

[12] B. A. Parker and T. R. Geissman, private communication.

[13] Compare with the infrared maxima of parthenin,[7] ambrosin,[7] and tenulin.[10]

methyl singlet, and the lactone ether proton resonance. The only way of incorporating all of these features in a proposed formula is to place the tertiary methyl group at the γ-carbon atom as in partial structure C (for VII) and D (for pulchellin). Ozonolysis of VII led in good yield to a lactol VIII (*cf.* similar results in the tenulin series[10,14]) whose reactions (positive Tollens test) corroborated partial structure C.[15]



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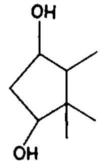

D

Oxidation of diacetylnorpulchellone (IVa) with potassium permanganate under controlled conditions led to the gummy diacetate of an α-ketol (IX). The infra-red spectra of IX and its transformation products (see Experimental) preclude their formulation as derivatives of cyclopentanone. Hence the second ring of pulchellin must be closed to C_1 of D.

A more direct proof, which coincidentally also established the location of the γ-lactone function on the bicyclo(5,3,0)decanediol template, was obtained in the following way. Treatment of IX with methanolic base,[17] or oxidation of IVa followed by hydrolysis without isolation of intermediates, led, after acidification, to the isolation of a substance $C_{12}H_{18}O_5$ (Xa, henceforth referred to as the lactone acid) which was the monolactone of a dihydroxydicarboxylic acid and had infra-red bands at 3500 and 3210 (nonbonded and bonded hydroxyl), 1750 (γ-lactone), and 1700 cm^{-1} (carboxyl). Conversion to a methyl ester (Xb) and a methyl ester acetate (Xc) which exhibited the appropriate infra-red maxima confirmed these assignments. Oxidation of Xb led to the ketolactone methyl ester XI, which was clearly a cyclopentanone derivative and had infra-red maxima at 1780 (γ-lactone) and 1740 cm^{-1} (combination of acetate, methyl ester, and cyclopentanone).

The spontaneous lactonization observed in the above reaction sequence demonstrates that one of the two carboxyl groups formed by cleavage of the α-ketol IX is *gamma* to one of the hydroxyl groups of pulchellin. Moreover, since the hydroxyl group involved is attached to a five-membered ring, the newly formed γ-lactone must be *cis*.

The relationship between the free carboxyl and the free hydroxyl group of the lactone acid was also easily determined. Treatment with acetic anhydride–sodium acetate converted X to a dilactone $C_{12}H_{16}O_4$ (XII) whose infra-red spectrum (bands at 1770 and 1735 cm^{-1}) showed that the newly formed lactone group was a δ-lactone. Hence four carbon atons intervene between the second hydroxyl group of pulchellin and the second carboxyl group formed by cleavage of the α-ketol.

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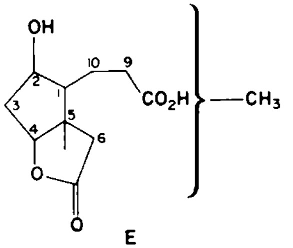
[14] D. H. R. Barton and P. de Mayo, *J. Chem. Soc.* **141**, (1956).

[15] Attempts to oxidize this lactol to an anhydride in the usual way met with failure. The infra-red spectrum of the crude product contained weak anhydride bands, but consisted mainly of starting material. Similar observations have recently been made on such lactols in the steroid field.[16]

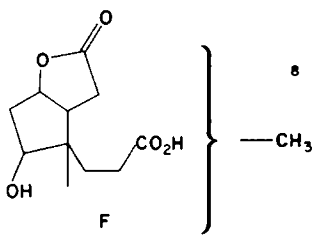
[16] R. Hirschmann, N. G. Steinberg, and R. Walker, *J. Amer. Chem. Soc.*, **84**, 1270 (1962); E. Caspi, W. Schmid, and B. T. Khan, *Tetrahedron*, **18**, 767 (1962).

[17] Air oxidation of an α-ketol in alkali, *cf.* M. Yanagita and A. Tahara, *J. Org. Chem.* **18**, 792 (1953). The oxidation proceeded with or without added perhydrol.

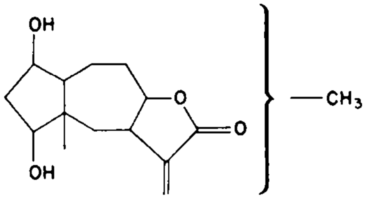
The chemical evidence presented in the preceding paragraphs limits possible structures for the lactone acid to two, i.e. E and F. We resist for the moment the



E



F



G

temptation to invoke the biogenetic isoprene rule which would of course settle the matter immediately in favor of E (methyl at C_{10}) and would lead directly to formula Ia for pulchellin.

Examination of the N.M.R. spectra of Table 1 clearly shows that E is correct. The argument is as follows. Ordinarily the H_2 resonance is found at somewhat lower field than that of H_4. This assignment is based on multiplicities, H_2 being spin coupled to three adjacent protons, H_4 to only two. In accordance with expectations the signal of H_2 is a complex multiplet. This multiplet, in the unacetylated compounds Ia and IIa is superimposed on the lactone ether hydrogen resonance but moves downfield on acetylation and can then be clearly discerned.

For reasons which are not immediately apparent, one of the H_3—H_4 coupling constants is generally zero, which gives the H_4 resonance the appearance of a doublet. However, the situation is much clearer in those compounds where strain imposed by lactone ring formation restores the expected multiplicity of H_4. In the N.M.R. spectrum of the lactone methyl ester Xb, for example, the H_2 resonance (complex multiplet) is superimposed on a doublet of doublets at 4·46 p.p.m. which must be ascribed to H_4 because of its multiplicity and which, because of its chemical shift (relative to I and II), must be attached to the carbon atom carrying the lactone oxygen. Acetylation to Xc moves the H_2 resonance, now clearly spin coupled to three protons, to much lower field, thus bringing into clear view the signal of H_4 which is coupled to two protons. Conversion of the lactone acid Xa to the dilactone XII again produces the shift in the H_2 signal expected on the basis of E.

Structure E for the lactone acid requires structure G for pulchellin. In assigning a locus to the secondary methyl group, we immediately exclude C_9 because of the complex N.M.R. signal produced by H_8. However, at this time there is no chemical basis for deciding between C_6 and C_{10} although on biogenetic grounds there is no alternative to formulating pulchellin as Ia. This problem is receiving further attention as is the stereochemistry. As regards the latter, the conversion of II to a cyclic sulphite

(XIV) and a carbonate (XV) clearly shows that the two hydroxyl groups are *cis* to each other and, because of the formation of Xa, *cis* to the C_5—C_6 side chain. If the Hudson-Klyne rule may be applied to X and XII ($M_{[d]}$ of XII—$M_{[d]}$ of Xa or Xb negative), the absolute configuration of the C_4-hydroxyl is *alpha* and the centers at C_2 and C_5 are therefore determined also.

EXPERIMENTAL[18]

Isolation of pulchellin. Gaillardia pulchella Foug. was collected near Mayport, Florida, in June 1958, 1959, and 1961 and near Caswell Beach, North Carolina, in June 1958. The crushed whole plant, wt. 145 lbs., was extracted with chloroform and worked up in the usual way,[7] wt. of crude gum 658 g. A 50 g portion was taken up in 200 ml of benzene–chloroform (1:3) and chromatographed on 400 g of alumina (Alcoa F-20) deposited with benzene. The chromatogram was successively eluted with 500 ml of benzene–chloroform (1:3), 500 ml of chloroform and chloroform–methanol (9:1) in 100 ml fractions. The benzene–chloroform eluate gave gummy material which did not crystallize; the chloroform eluate gave gummy material which solidified on trituration with ether. Elution with chloroform–methanol gave crude crystalline pulchellin directly, total yield of crude pulchellin from 145 lbs of whole plant 112 g.

Crude crystalline pulchellin, wt. 20 g was dissolved in 400 ml of benzene–chloroform (1:3) containing a small amount of methanol and rechromatographed over 300 g of alumina (Merck, acid-washed). Chloroform eluted nothing. Chloroform–methanol (8:2) eluted 17 g of pulchellin which was recrystallized from ethyl acetate, benzene, or water, m.p. 165–168°, $(\alpha)_{d}^{26}$ $-36{\cdot}21°$ ($CHCl_3$, *c* 2·43), λ_{max} 209 mμ (ε 9400), infrared bands at 3560 (hydroxyl), 1763 (γ-lactone), and 1660 cm^{-1} (double bond). (Found: C, 67·30; H, 8·37; Calcd. for $C_{15}H_{22}O_4$: C, 67·64; H, 8·33).

Mother liquors from the recrystallization of crude pulchellin (from the chloroform–methanol eluate) were rechromatographed. The last methanol eluate afforded crystals, m.p. 130–160°, which were recrystallized from acetone–pentane, m.p. 163–165°, depression on admixture of pulchellin. Infra-red spectrum and mixed m.p. established identity with 12-methoxypulchellin (IIIa, *vide infra*).

On ozonolysis in the usual manner, pulchellin gave the dimedone derivative of formaldehyde, m.p. and mixed m.p. 181°.

Diacetylpulchellin (Ib). The acetate was prepared by the acetic anhydride–pyridine method using 0·9 g of pulchellin, 6 ml of acetic anhydride, and 3 ml of pyridine, yield 1·2 g, m.p. 121–123° after one crystallization from benzene–petroleum ether. This material was sufficiently pure for the oxidations to be described subsequently. Chromatography over alumina and crystallization from benzene–petroleum ether raised the m.p. to 123–125°, $(\alpha)_{D}^{25}$ $-28{\cdot}96°$ ($CHCl_3$, *c* 1·83), infra-red bands at 1770(γ-lactone, 1735) and 1240 (acetate) and 1660 cm^{-1} (double bond). (Found: C, 65·43; H, 7·28; Calcd. for $C_{19}H_{26}O_6$: C, 65·12; H, 7·48).

A mixture of 0·2 g of diacetylpulchellin and 4 ml of 5% methanolic potassium hydroxide solution was allowed to stand at room temperature for 24 hr, acidified with dilute hydrochloric acid and extracted with chloroform. The chloroform layer after washing and drying furnished 0·08 g of solid, m.p. 139–148°, which was recrystallized from ethyl acetate and shown to be identical with IIIa by mixed m.p. and infra-red spectrum.

12-*Methoxypulchellin* (IIIa). A mixture of 0·5 g of pulchellin and 10 ml of 5% methanolic potassium hydroxide was kept at room temperature overnight and worked up as described in the preceding paragraph, yield 0·45 g, m.p. 135–139°. It was taken up in benzene–chloroform and chromatographed over alumina. The chloroform eluate was recrystallized from benzene and then from acetone–pentane, yield 0·17 g, m.p. 163–165°, m.p. depression on admixture of pulchellin, $(\alpha)_{D}^{29}$ $-15{\cdot}7°$ ($CHCl_3$, *c* 1·85), infra-red maxima at 3450 (hydroxyl, 1770 (γ-lactone), 1085 cm^{-1} (methoxyl). (Found: C, 64·55; H, 8·44; Calcd. for $C_{16}H_{26}O_5$: C, 64·40; H, 8·78).

12-Methoxypulchellin was also formed by contact of pulchellin with basic alumina in methanol or on hydrolysis of diacetylpulchellin. It was acetylated by treatment with acetic-anhydride-pyridine and crystallized from water, total yield from 0·3 g of starting material 0·38 g (this included working up

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[18] M.p.'s are uncorrected. Analyses by Dr. F. Pascher, Bonn, Germany. Infra-red spectra were taken in chloroform solution, unless otherwise specified, on a Perkin-Elmer Infracord spectrophotometer. Ultra-violet spectra were run by Mrs. P. DeTar and Mrs. P. Ward on a Cary model 14 spectrophotometer in 95% ethanol solution.

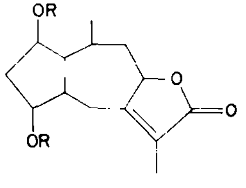
the aqueous mother liquors), m.p. 132–135°, (α)$^{29}_{D}$ −4·6° ($CHCl_3$, *c* 1·74), infrared bands at 1775 (γ-lactone), 1740, 1240, and 1050 cm^{-1} (acetate). (Found: C; 63·06; H, 7·91; O, 29·10; Calcd. for $C_{20}H_{30}O_7$: C, 62·81; H, 7·91; O, 29·29).

The diacetate could be hydrolyzed to IIIa by treatment with aqueous-methanolic potassium hydroxide. An attempt to convert IIIa to pulchellin by allowing it to stand with a solution of acetic acid containing hydrochloric acid resulted in conversion to IIIb, yield 65%.

Dihydropulchellin (IIa). A solution of 1·0 g of pulchellin in 150 ml of 95% ethanol was reduced with 0·08 g of prereduced platinum oxide at atmospheric pressure, observed hydrogen uptake 94 ml, calcd. for one double bond 94 ml. The solvent was removed and the residue recrystallized repeatedly from acetone–petroleum ether. The colourless needless melted at 136–138°, (α)$^{28}_{D}$ −0·61° ($CHCl_3$, *c* 1·64), yield 0·7 g, infra-red bands at 3400 (hydroxyl) and 1760 cm^{-1} (γ-lactone). (Found: C, 67·26; H, 8·88; Calcd. for $C_{15}H_{24}O_4$: C, 67·13; 9·02 g).

Reduction of pulchellin with palladium–charcoal or palladium-on-calcium carbonate gave IIa in 60–80% yield accompanied by isopulchellin, (XVIa) the relative amount of which varied on the experimental conditions. In a typical experiment, 3 g of pulchellin in 120 ml of methanol was hydrogenated with 0·8 g of 5% palladium charcoal. The viscous residue was crystallized from acetone–pentane. Four crops of IIa, total weight 1·85 g (62%) were collected. The mother liquor afforded two crops of m.p. 118–121°, wt. 0·3 g and 134–136, wt. 0·2 g, after being kept in the refrigerator for one and two months. Recrystallization from acetone–pentane furnished prisms of XVIa, m.p. 138–141° (depression on admixture of IIa), (α)$^{29}_{D}$ −12·98° ($CHCl_3$, *c* 2·28), λ_{max} 221 mμ (ε 10350), infra-red bands at 3450 (hydroxyl), 1745, and 1670 cm^{-1} (α,β-unsaturated γ-lactone). (Found: C, 66·04; H, 9·20; O, 24·94; Calcd. for $C_{15}H_{22}O_4{\cdot}C_3H_6O$; C, 66·64; H, 8·70; O, 24·66).

The diacetate, XVIb, melted at 147–149°. (Found: C, 64·38, H, 8·37; O, 27·18: Calcd. for $C_{19}H_{28}O_6$: C, 64·75; H, 8·01; O, 27·24).



XVIa R=H
b R=Ac

Diacetyldihydropulchellin (IIb). Treatment of IIa with acetic anhydride–pyridine furnished solid material which was recrystallized repeatedly from acetone–pentane, m.p. 155–157°, (α)$^{28}_{D}$ +4·86° ($CHCl_3$, *c* 1·44), infrared bands at 1770 (γ-lactone), 1735, 1240, and 1050 cm^{-1} (acetate). (Found: C, 64·61; H, 7·85; Calcd. for $C_{19}H_{28}O_6$: C, 64·75; H, 8·01).

This substance was also prepared by hydrogenation of Ib with platinum oxide in ethanol. Saponification with 5% methanolic hydroxide solution at room temperature for 20 hr followed by acidification and extraction with chloroform furnished IIa.

Attempts to prepare ethylidene, p-nitrobenzylidene and benzylidene derivatives, and an acetonide of IIa resulted in 35–60% recovery of starting material. Attempts to prepare a ditosylate resulted in recovery of 50–60% of IIa, or noncrystallizable gums. IIa reacted with trityl chloride to give unsharply melting material whose analysis corresponded to that of mixtures of mono- and ditrityl ethers.

The dimesylate IIc was prepared from 0·3 g of IIa, 0·35 g methanesulfonyl chloride, and 4 ml pyridine. The crude product was recrystallized from acetone–pentane, yield 0·3 g, m.p. 137°. (Found: C, 47·86; H, 6·52; O, 30·23; S, 15·41; Calcd. for $C_{17}H_{28}O_8S_2$: C, 48·09; H, 6·65; O, 30·11; S, 15·11).

Sulphite of dihydropulchellin (XIV). To a solution of IIa, wt. 0·05 g in 1 ml of pyridine was added with cooling three drops of thionyl chloride. After one hour at room temperature, the mixture was poured onto ice water and ether. The solid which separated was washed with water and ether and dried. The amorphous material, wt. 0·045 g, was recrystallized from acetone–pentane and chloroform–methanol, m.p. 189–191° (dec.), infra-red bands at 1780 (γ-lactone) and 1197 cm^{-1} (sulphite). (Found: C, 56·79; H, 7·20; O, 25·66; S, 10·56; Calcd. for $C_{15}H_{22}O_6S$: C, 57·28; H, 7·06; O, 25·46; S, 10·71).

Hydrolysis of 0·02 g of the sulphite with 5 % methanolic potassium hydroxide at room temperature for three days, removal of solvent, neutralization and extraction with acetone furnished material identical with IIa.

Carbonate of dihydropulchellin (XV). Treatment of 0·1 g of IIa in pyridine with phosgene in the usual way furnished a gum which was dissolved in benzene and chromatographed over 10 g of acid-washed alumina. Chloroform eluted amorphous material which was recrystallized from acetone–ether, but remained amorphous, m.p. 210–215° (dec.), infra-red bands (nujol) at 1770 (γ-lactone), 1740, 1265, and 1000 cm^{-1} (carbonate). (Found C, 64·71; H, 8·00; Calcd. for $C_{16}H_{22}O_5 \cdot \frac{1}{4}H_2O$: C, 64·32; H, 7·61).

Dehydrodihydropulchellin (VIa). A solution of 1 g of IIa in 10 ml of pyridine was added dropwise with stirring to pyridine–chromium trioxide complex prepared from 1 g of CrO_3 and 20 ml of pyridine. After 2·5 hr at room temperature (longer stirring reduced the yield) the mixture was poured into ice and the product extracted with ether. The ether extract was washed, dried, and evaporated and the crystalline residue recrystallized from acetone–ether, yield 0·46 g, m.p. 185–190°. Further purification was effected by chromatography over acid-washed alumina (eluent–chloroform 3:7) and repeated crystallization from acetone-benzene, m.p. 189–190°, $(\alpha)^{29}_{P}$ +74·18° ($CHCl_3$, *c* 4·26), infra-red bands at 3500 (hydroxyl), 1775 (γ-lactone), and 1740 cm^{-1} (cyclopentanone). In subsequent runs the yields were 45–55 %. The substance gave positive Tollens and Zimmermann tests. (Found: C, 67·96; H, 8·19; Calcd. for $C_{15}H_{22}O_4$: C, 67·74; H, 8·33).

The acetate (VIb) prepared by the acetic anhydride–pyridine method, crystallized from acetone–pentane as colourless pillars, m.p. 182–185°. The Tollens test was negative, the Zimmermann test positive. (Found: C, 66·20; H, 7·75; Calcd. for $C_{17}H_{24}O_5$: C, 66·21; H, 7·85).

The mesylate (VIc) was prepared from 0·46 g of VIa and 0·3 g of methanesulphonyl chloride in 2 ml of pyridine. Recrystallization from acetone–pentane afforded small prisms, m.p. 132–134°, infra-red bands at 1770 (γ-lactone), 1747 (cyclopentanone), and 1180 cm^{-1} (sulphonate). When attempts were made to prepare an analytical sample by drying in the usual way, the material gradually underwent a change, passing through an oily intermediate into solid prisms, m.p. 127–130°, which were shown to be identical with VII (*vide infra*), by mixed m.p. and infra-red spectrum.

Anhydrodehydrodihydropulchellin (VII). This was prepared from VIc by treatment with lutidine or sodium acetate in ethanol or directly from VIa with methanolic potassium hydroxide. The mesylate, wt. 1 g, was refluxed with 2,6-lutidine in a nitrogen atmosphere, cooled, poured onto ice water, and the mixture acidified to pH 6. The precipitate was filtered and the filtrate extracted with chloroform. The organic extract was washed thoroughly, dried, and evaporated. The oily residue was combined with the solid (total wt. 0·93 g), dissolved in benzene, and chromatographed over neutral alumina. The benzene eluates were recrystallized from acetone–pentane, yield 0·45 g, m.p. 160–163° Benzene–chloroform (9:1) eluted an additional 0·14 g of product. Further crystallization gave pillars, m.p. 162–163° $(\alpha)^{27}_{D}$ +34·73° ($CHCl_3$, *c* 2·39), infra-red bands at 1770 (γ-lactone), 1705, and 1595 cm^{-1} (cyclopentenone), λ_{max} 220 mμ (ε 10300). (Found: C, 72·53; H, 7·81; O, 19·35; Calcd. for $C_{15}H_{20}O_3$ C, 72·55; H, 8·12; O, 19·33).

Yields of subsequent runs ranged from 54–78 %. Treatment of 0·1 g of the mesylate with 0·1 g of fused acetate in 5 ml of ethanol at reflux for 12 hr, removal of solvent and recrystallization yielded material melting at 127–130° whose infra-red and U.V. spectra were superimposable on those of VII. The m.p. difference is probably due to dimorphism. (Found: C, 72·52; H, 8·30; O, 19·52; Calcd. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33).

Ozonolysis of VII. A solution of 0·45 g of VII in 25 ml of chloroform was ozonized at −10° for 1·75 hr. The mixture was shaken with water for 1 hr and allowed to stand overnight, the organic layer separated, washed, and evaporated. The residual gum was stirred with 50 ml of sodium bicarbonate solution for 10 hr, upon which treatment it gradually changed to a crystalline solid which was collected, washed with water, and dried. The bicarbonate solution on acidification yielded additional product, total yield 0·28 g (neutral and acidic fraction exhibited the same infra-red spectrum). An additional 0·05 g of material was obtained from the filtrate after evaporation to dryness and extraction with chloroform, total yield 0·33 g (86 %). Recrystallization from aqueous methanol afforded needle-like prisms, m.p. 132–205°, positive Tollens test. After drying at room temperature *in vacuo* for several days, the m.p. was 66–70°, $(\alpha)^{28}_{D}$ +2·09° ($CHCl_3$, *c* 2·04), $(\alpha)^{28}_{D}$ +0·85° (95 % ethanol, *c* 1·53), infra-red maxima at 3550 (free —OH), 3300 (bonded —OH), and 1775 (lactones). (Found: C, 62·48; H, 8·07; O, 29·87; Calcd. for $C_{14}H_{20}O_5$: C, 62·67; H, 7·51; O, 29·82).

Dihydroanhydrodehydrodihydropulchellin (XIII). A solution of 0·24 g of VII in 50 ml of 95% methanol was reduced with 0·05 g of 5% palladium charcoal at atmospheric pressure, hydrogen uptake observed 28 ml, calcd. for one double bond 24·5 ml. Removal of solvent gave colourless prisms, m.p. 136–139°, in quantitative yield, which were recrystallized from acetone–pentane, m.p. 146–148°, $(\alpha)^{28}_{P}$ $-129{\cdot}12°$ ($CHCl_3$, *c* 1·82), infra-red bands at 1770 (γ-lactone), and 1740 cm^{-1} (cyclopentanone). (Found: C, 71·89; H, 8·78; O, 19·35; Calcd. for $C_{15}G_{22}O_3$: C, 71·97; H, 8·86; O, 19·17).

Diacetylnorpulchellone (IVa). A solution of 1·0 g of diacetylpulchellin in 23 ml of methanol was ozonized at −78° until the solution showed the characteristic blue colour. The solution was steam distilled. Crystals began to separate. After chilling, the product, wt. 0·82 g, was filtered, washed with water, and dried, m.p. 200–205, yield 80–93%. Recrystallization from acetone–pentane furnished colourless plates, m.p. 213–215° (dec.), positive ferric chloride test, λ_{max} 237 mμ (ε 9800), infra-red bands at 3540 (nonbonded —OH), 3150 (bonded —OH), 1760 (γ-lactone-shoulder), 1740 (acetate), and 1665 cm^{-1} (conjugated double bond). (Found: C, 61·54; H, 7·31; Calcd. for $C_{18}H_{24}O_7$: C, 61·35; H, 6·86).

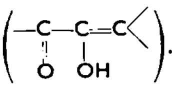
Norpulchellone triacetate (IVb) was prepared from 0·8 g of IVa, 10 ml of isopropenyl acetate, and a trace of p-toluenesulphonic acid by refluxing for 3 hr and cooling. The solvent was removed *in vacuo*, and the residue neutralized with sodium bicarbonate and extracted with chloroform. The organic layer was washed, dried, and concentrated. The residual gum was dissolved in benzene and chromatographed over neutral alumina. Benzene–chloroform (7:3) eluted prisms, wt. 0·68 g, which melted at 148–149° after crystallization from acetone–pentane. (Found: C, 61·38; H, 7·11; O, 31·84; Calcd. for $C_{20}H_{26}O_8$: C, 60·90; H, 6·64; O, 32·45).

Oxidation of IVa. To a mixture of 3 g of diacetylnorpulchellone and 3 g of magnesium sulphate in 150 ml of purified acetone was added dropwise with stirring at a temperature below 0° a solution of 2·25 g of potassium permanganate (equivalent to 2·5 atoms of oxygen) in 75 ml of water and 75 ml of acetone over a period of 3 hr. Stirring was continued below 0° for 8 additional hours until the colour of potassium permanganate had disappeared. The mixture was warmed to steam bath temperature, filtered, the precipitate washed with acetone, and the combined filtrate and washings evaporated at reduced pressure. The residual yellow gum consisted mainly of IX and a little Xa. It was taken up in chloroform, extracted with sodium bicarbonate solution, washed, dried, and evaporated. The residual gum (IX) could not be induced to crystallize, wt. 2·0 g, infra-red maxima at 3600 (hydroxyl), 1740, and 1240 (acetate), and 1700 cm^{-1} (cycloheptanone), periodic acid and Tollens tests positive. It was converted to a dinitrophenylhydrazone in the usual way, yield 0·065 g of orange-yellow material from 0·068 g of gum. The derivative was purified by passage through an alumina column and afforded fine yellow needles after recrystallization from ethanol. (Found: C, 53·59; H, 6·11; N, 10·35; Calcd. for $C_{22}H_{28}O_9N_4{\cdot}C_2H_6O$: C, 53·52; H, 6·36; O, 10·40).

The acetate was prepared by the pyridine–acetic anhydride method. The product could not be induced to crystallize, b.p. 200–210° (0·1 mm, bath temperature), infra-red maxima (carbon tetrachloride) at 1745 and 1240 cm^{-1} (acetate). Satisfactory analyses could not be obtained. (Found: C, 59·59; H, 7·41; O, 32·97; Calcd. for $C_{18}H_{26}O_7$: C, 61·00; H, 7·40; O, 31·60).

The sodium carbonate extract on acidification to pH 6, evaporation *in vacuo*, and treatment of the residue with 10% hydrochloric acid afforded crystalline material which was recrystallized from water and shown to be identical with Xa (*vide infra*) by mixed m.p. and infra-red spectrum, yield 0·04 g.

A solution of 0·3 g of the α-ketol IX in 3 ml of 70% aqueous acetic acid and 0·3 g cupric acetate was refluxed for 10 min, cooled, diluted with water, and extracted with ether. The organic extract on washing and drying furnished the α-diketone as a pale-yellow viscous oil, positive ferric chloride test, λ_{max} 270 mμ (3000), infra-red bands at 3600 (hydroxyl), 1740, and 1240 (acetone), 1690 and 1650 cm^{-1}



$$\left(-\underset{\substack{\| \\ \mathrm{O}}}{\mathrm{C}}-\underset{\substack{| \\ \mathrm{OH}}}{\mathrm{C}}=\mathrm{C}\langle\right).$$

Oxidative hydrolysis of IX. A solution of 1·0 g of IX in 25 ml of 4% methanolic potassium hydroxide was allowed to stand at room temperature for 24 hr. The solution was acidified to pH_6 and evaporated *in vacuo* at room temperature. The residue was triturated with 10% hydrochloric acid, which caused crystallization. Recrystallization from water furnished 0·5 g (60%), of the lactone acid Xa, m.p. unsharply in the range 110–138° (evolution of water), highest m.p. 135–138°, $(\alpha)^{25}_{D}$ $+80{\cdot}10°$ (acetone, *c* 1·91), infra-red bands (KBr disk) at 3500 and 3210 (bonded and nonbonded hydroxyl), 1750 (γ-lactone, and 1700 cm^{-1} (carboxyl). Oxidation with alkaline perhydrol resulted in a 50% yield of purified material, m.p. 122–125°, identified as Xa by thin layer chromatography. (Found: C, 55·28; N, 7·56; O, 37·00; Calcd. for $C_{12}H_{18}O_5 \cdot H_2O$: C, 55·37; H, 7·75; O, 36·88).

The methyl ester (Xb) was prepared by suspending Xa in ether and adding an ethereal solution of diazomethane. Gradual solution took place. After 5 hr, the solution was filtered and evaporated and the solid residue recrystallized from acetone–pentane. The colourless needles melted at 82–84°, $(\alpha)_D^{28}$ +81·36° ($CHCl_3$, *c* 2·56), infra-red bands at 3500 (hydroxyl), 1765 (γ-lactone) and 1730 cm^{-1} (ester carbonyl). (Found: C, 60·80; H, 7·78; O, 31·07; Calcd. for $C_{13}H_{20}O_5$: C, 60·92; H, 7·87; O, 31·21)

The acetate Xc was prepared from Xb by the acetic anhydride–pyridine method and recrystallized from ether–pentane, m.p. 74–76°, infra-red bands at 1775 (γ-lactone) and 1730 cm^{-1} (combination of acetate and methyl ester). (Found: C, 60·78; H, 7·31; O, 32·39; Calcd. for $C_{15}H_{22}O_6$: C, 60·39; H, 7·43; O, 32·18).

The tosylate Xa was prepared in the usual way and recrystallized from ether–pentane–chloroform and acetone–pentane, m.p. 113–115°, infra-red bands at 1775 (γ-lactone), 1730 (ester), 1175, and 1095 cm^{-1} (sulphonate). (Found: C, 58·17; H, 6·46; O, 27·42; S, 7·93; Calcd. for $C_{20}H_{26}O_7S$: C, 58·51; H, 6·39; O, 27·38; S, 7·80).

Oxidation of Xb. A solution of 0·33 g of Xb in 25 ml of acetic acid was mixed with 0·13 g of chromium trioxide in 25 ml of 95% acetic acid and allowed to stand in the refrigerator for 8 hr. Excess reagent was destroyed with methanol and the solution evaporated to dryness at reduced pressure. The residue was extracted with ether and the ether layer washed, dried, and concentrated. The oily residue (XI, yield 65%) could not be induced to crystallize, b.p. 160–170° (0·5 mm, bath temperature), infra-red bands at 1780 (γ-lactone) and 1750 cm^{-1} (combination of cyclopentanone and ester carbonyl) positive Zimmermann test. (Found: C, 60·90; H, 7·23; O, 31·61; Calcd. for $C_{13}H_{18}O_5$: C, 61·40; H, 7·14; O, 31·46).

Lactonization of Xa. A solution of 0·2 g of Xa and 0·06 g of sodium acetate in 15 ml of acetic anhydride was refluxed for 5 hr and evaporated to dryness *in vacuo*. The residue was extracted with ether and the ether extract washed, dried, and evaporated. The residue was taken up in benzene and chromatographed over acid-washed alumina. Benzene-chloroform eluted 0·09 g of the dilactone XII, which was recrystallized from acetone–pentane. The colourless needles melted at 142–143°, $(\alpha)_D^{28}$ +7·65° $CHCl_3$, *c* 1·83), infra-red bands at 1770 (γ-lactone) and 1735 cm^{-1} (δ-lactone). (Found: C, 64·48; H, 7·10; O, 28·36; Calcd. for $C_{12}H_{16}O_4$: C, 64·27; H, 7·19; O, 28·54).

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