Phytochemistry, 1968, Vol. 7, pp. 1613 to 1621. Pergamon Press. Printed in England

SESQUITERPENE LACTONES. CONSTITUENTS OF DIPLOID AND POLYPLOID *AMBROSIA DUMOSA* GRAY[1]

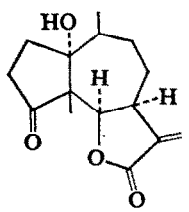
T. A. GEISSMAN and SUMU MATSUEDA

Department of Chemistry, University of California, Los Angeles 90024

(*Received* 20 *March* 1968)

Abstract—Diploid and polyploid forms of *Ambrosia dumosa* Gray have been found to differ markedly in chemical constitution. From a diploid form were isolated four known lactones: coronopilin, ambrosiol, psilostachyin and parthenolide. From a polyploid form were isolated psilostachyin C and parthenolide; and two new lactones, burrodin and apoludin, the structures of which have been established. Parthenolide is the only identifiable lactonic constituent that is found in both forms.

Ambrosia dumosa Gray (family Compositae, tribe Ambrosieae), a common perennial of the deserts of the western United States, occurs in a wide range of forms differing in chromosome number and in morphology.[2] Earlier studies on the diploid (the commonest) form have been referred to,[3] when it was observed that the plant contains coronopilin (I), a compound widely distributed in the ragweeds. We have now made a more detailed examination of *A. dumosa* specimens of both diploid and polyploid forms, and find that they show marked differences in chemical constitution.

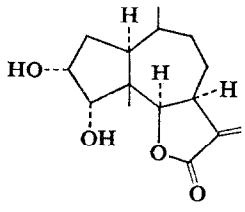


(I)

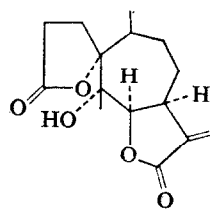
The diploid form of *A. dumosa* Gray is typically a low, rounded, gray-green shrub, often scarious; but in favorable conditions of adequate rainfall it bears a profusion of small (1–2 cm), delicate leaves twice or thrice pinnately parted and a short spicate inflorescence. The polyploid form[4] used in this study was a larger, darker green plant with coarse bipinnate leaves and a much larger inflorescence. The material from the two forms used in this work was the mixed leaves and flower spikes, stripped from the woody stems.

[1] Contribution No. 2224 from the Department of Chemistry, U.C.L.A.
[2] W. W. PAYNE, P. A. RAVEN and D. W. KYHOS, *Am. J. Botany* **51**, 419 (1964).
[3] T. A. GEISSMAN and R. W. TURLEY, *J. Org. Chem.* **29**, 2553 (1964).
[4] Professors Payne and Raven have examined the pollen grains of the sample used in this work and have concluded that the plant is probably tetraploid ($n=36$), but possibly hexaploid ($n=54$). It was not possible to procure material suitable for meiotic chromosome counts.

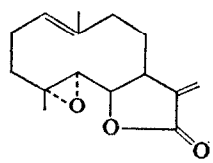
From the diploid *A. dimosa* were isolated four lactones, all of them previously known, three of them from other *Ambrosia* species. They are coronopilin (I), ambrosiol (II),[5] psilostachyin (III),[6] and parthenolide (IV).[7] Parthenolide is of special interest, for its only reported occurrence in the Compositae prior to this work is in *Chrysanthemum parthenium*, a member of the tribe Anthemideae. All of these compounds were identified by direct comparison of their physical and chemical properties with those of authentic specimens. It should be noted that extracts of the diploid plant show about nine distinct components on thin-layer chromatograms, two or three of which appear to be coumarins.



(II)

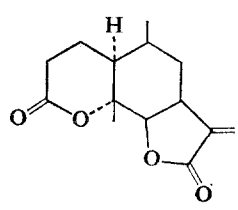


(III)

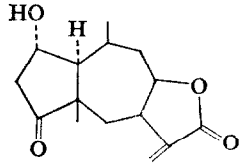


(IV)

The polyploid *A. dumosa* also contained parthenolide, which is the only isolable compound common to both forms.[8] In addition to this compound there were found psilostachyin C (V), and two new lactones, named burrodin (VI) and apoludin (XII).



(V)

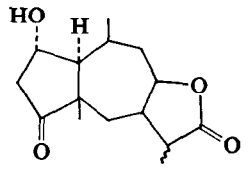


(VI)

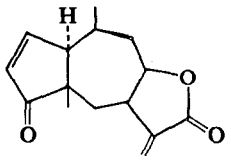
Burrodin (VI), m.p. 167–168°, was obtained in 0·3 per cent yield from the dried polyploid plant material. It has the composition $C_{15}H_{20}O_4$ and shows the molecular ion M^+ 264 in the mass spectrometer. Its i.r. and u.v. spectra (see Experimental) suggested that it was an α-methylene-γ-lactone, and this was borne out by the NMR spectrum, which showed the pair of one-proton doublets (δ 5·71 and 6·33, J=2·5 c/s) characteristic of this structural grouping. The intensity and position (1760 cm^{-1}) of the broad carbonyl peak in the i.r.

[5] T. J. MABRY, W. RENOLD, H. E. MILLER and H. B. KAGAN, *J. Org. Chem.* **31**, 681 (1966).
[6] T. J. MABRY, H. E. MILLER, H. B. KAGAN and W. RENOLD, *Tetrahedron* **22**, 1139 (1966).
[7] T. R. GOVINDACHARI, B. S. JOSHI and V. N. KAMAT, *Tetrahedron Letters* 3927 (1964).
[8] Careful examinations of thin-layer chromatograms shows that only parthenolide is common to both diploid and polyploid forms, no other constituent in one form being seen on the chromatogram of the other.

spectrum indicated that it represented the combined absorption of the lactone and a cyclopentanone ring. Other NMR signals were seen at δ 1·05 (3H, singlet, C-5 methyl); 1·22 (3H, doublet, $J=7$ c/s, C-10 methyl); and a complex multiplet at δ 4·5–4·9 (2H, lactone C*H*—O and CHOH). Catalytic reduction of burrodin yields a dihydro compound; the appearance of a new three-proton signal at δ 1·20 (doublet, $J=7·5$ c/s) and the disappearance of the pair of doublets at δ 5·7 and 6·3 shows that the exocyclic methylene group has been reduced, with the formation of 11,13-dihydroburrodin (VII).

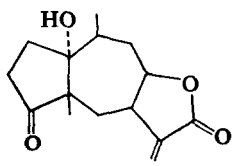


(VII)

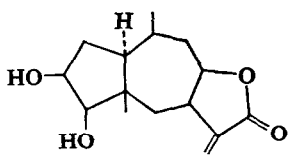


(VIII)

Burrodin was easily dehydrated under mild conditions by means of methanesulfonyl chloride and pyridine. Anhydroburrodin (VIII) shows the u.v. and i.r. spectral characteristics of a conjugated cyclopentenone, and its constitution as the cyclopent-2-en-4-one is clearly shown by the NMR spectrum. The protons at C-2 and C-3 appear as a pair of doublets at δ 6·08/6·18 and δ 7·52/7·62, each being further split by the proton at C-1. The signals at 6·08/6·18 (C-3) are doublets with $J=3$ c/s, and those at 7·52/7·62 (C-2) are doublets with $J=1$ c/s. The proton of the C*H*—O grouping of the lactone appears as a broad one-proton signal with a multiplicity of peaks at δ 4·60, 4·66, 4·74, 4·78, 4·85 and 4·92, an indication that the lactone is closed to the C-8 position. The o.r.d. curve of burrodin shows a positive Cotton effect, similar in amplitude to those of such other lactones as peruvin (IX)[9] and cumanin (X)[10] indicating that the 5/7 ring junction is *trans*. Final confirmation of the position and stereochemistry of the lactone function was obtained by the preparation of tetrahydroanhydroburrodin (XI), m.p. 146–147°, $[\alpha]_D^{28}$ +121°, evidently the same as the compound obtained from cumanin.[10,11] Since cumanin has been correlated with peruvin,[9] and peruvin with mexicanin A and helenalin,[12,13] the stereochemistry of the lactone ring in burrodin is as shown in VI. The final stereochemical detail of burrodin, the configuration of the hydroxyl group at C-2, was established by the use of the Horeau method, which showed that this hydroxyl group is α-oriented.[14,5]



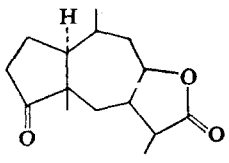
(IX)



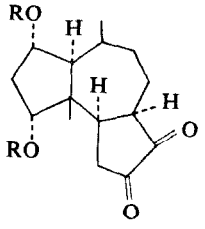
(X)

[9] P. Joseph-Nathan and J. Romo, *Tetrahedron* **22**, 1723 (1966).
[10] J. Romo, P. Joseph-Nathan and G. Siade, *Tetrahedron* **22**, 1499 (1966).
[11] Reported for cumanin-derived lactone, m.p. 147–8°, $[\alpha]_D+128°$.[10]
[12] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *J. Am. Chem. Soc.* **85**, 19 (1963).
[13] M. T. Emerson, C. N. Caughan and W. Herz, *Tetrahedron Letters* 621 (1964).
[14] A. Horeau, *Tetrahedron Letters* 506 (1961); A. Horeau and H. B. Kagan, *Tetrahedron* **20**, 2431 (1964).

Apoludin (XII), m.p. 134–135°, was obtained in 0·19 per cent yield from the polyploid plant. It has the composition $C_{15}H_{22}O_4$ (M^+ observed 266), and gave i.r. and u.v. spectra which showed the presence of the α-methylene-γ-lactone grouping and the absence of other carbonyl functions. The NMR spectrum showed the protons of the exocyclic methylene group at δ 5·52 and 6·24 ($J=3$ c/s), and signals at δ 1·10 (3H, singlet, C-5 methyl), 1·36 (3H, doublet, $J=7$ c/s, C-10 methyl), 3·95 (1H, triplet, $J=8$ c/s, C*H*—OH at C-4), 4·37(1H, multiplet, C*H*—OH at C-2), and 4·39 (1H, doublet, $J=9{\cdot}5$ c/s). The latter signal, characteristic of the C-6 proton of the *cis*-fused lactones of the group exemplified by coronopilin, ambrosin, parthenin and ambrosiol, indicates that apoludin belongs to this class of lactones. The spectral evidence suggests that apoludin is the 2,4-diol (XII) of structure corresponding in other respects to ambrosiol, the 3,4-diol (II).

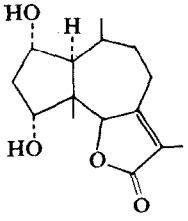


(XI)

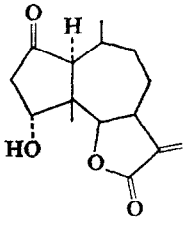


(XII), R=H
(XIII), R=Ac

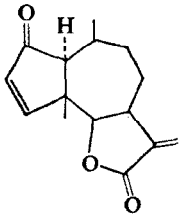
Apoludin yields a diacetate (XIII), in which the protons of the C*H*—OAc groupings are seen at δ 5·07 (1H, triplet, $J=9$ c/s) and 5·2–5·3 (1H multiplet). This observation confirms the presence of two secondary hydroxyl groups in apoludin. When an attempt was made to hydrogenate apoludin, isomerization of the exocyclic double bond occurred with the formation of isoapoludin (XIV), the NMR spectrum of which showed the new C-11 methyl group at δ 1·78 as a three-proton singlet and the disappearance of the downfield signals of the exocyclic methylene group of apoludin. The lactonic proton, which appears as a doublet at δ 4·39 in apoludin, is seen in isoapoludin as a one-proton singlet at δ 4·54, in accord with the structure XIV.



(XIV)



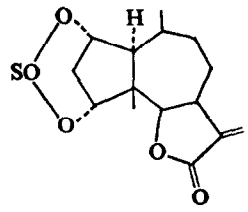
(XV)



(XVI)

The hydroxyl group at C-2 of apoludin was readily oxidized by chromic acid with the formation of 2-dehydroapoludin (XV), which was readily dehydrated to yield anhydrodehydroapoludin (XVI). The NMR signals for the protons at C-3 and C-4 are doublets (δ 6·08 and 7·75, $J=6$ c/s), in contrast to those of the protons at C-2 and C-3 of anhydroburrodin, whose NMR signals show the additional coupling with the proton at C-1. The lactonic proton at C-6 of anhydrodehydroapoludin appears as the doublet (δ 5·08, $J=9$ c/s) characteristic of the *cis*-fused C-6/C-7 lactone.

Apoludin forms a cyclic sulfite (XVII), demonstrating that the hydroxyl groups at C-2 and C-4 are *cis*-disposed. Application of the Horeau method to 2-dehydroapoludin showed that the hydroxyl group at C-4 is α-oriented and thus that the structure of apoludin is represented by XII.



(XVII)

DISCUSSION

The taxonomic implications of the wide disparity between the chemical constitution of diploid and polyploid forms of a single taxon cannot be fully assessed until more information on the chemical consequences of polyploidy can be assembled by further studies. Indeed, although Payne[2] commented that "plants of *Franseria* (=*Ambrosia*) *dumosa* at the diploid vs. polyploid levels appear strikingly different in the field, in morphology as well as time of blooming and ecological preferences.[15] It appears likely that further study will result in taxonomic subdivision of this complex", more recent investigations[16] of the morphology and cytology of the complex indicates an overall lack of correlation which makes such a distinction impossible. In view of the recognized variability within groups of plants characterized as belonging to single species[17] it will be necessary to enlarge the studies on *A. dumosa* in order to determine whether the chemical variability described here is a reflection of intraspecific variation or is uniquely associated with differences in chromosome number.

In our view, the most significant result from this study is the discovery of parthenolide[18] as the only constituent common to both the diploid and polyploid forms. Although the details of the biosynthesis of the sesquiterpenoid lactones are not known in detail, their formation from farnesyl pyrophosphate by way of a germacranolide progenitor is a generally accepted conjecture. It will be apparent that parthenolide (IV) is the 4,5-epoxide formed from the cyclodecadiene lactone (i.e. costunolide) that lies near the beginning of this presumptive biosynthetic pathway.[19] The presence of parthenolide in the two forms studied here supports the supposition that it is an early precursor which lies at one end of a pathway which, under the genetic controls of the two forms, suffers diversion in a number of ways as structural elaboration proceeds.

It is pertinent to note that Payne, *et al.*[2] have also called attention to the apparent relationships between the Ambrosieae and the Anthemideae, for parthenolide is so far known to

[15] We note, however, that at the time of our collections diploid and polyploid forms were growing side by side and were in substantially the same stage of floral development.

[16] W. W. PAYNE, personal communication.

[17] T. J. MABRY (*Ambrosia confertiflora*), reported at the Second Natural Products Symposium, Mona, Jamaica, January 3–5, 1968; T. J. MABRY (*Ambrosia psilostachya*), personal communication; unpublished observations in this laboratory (*Xanthium strumarium*, *Ambrosia acanthicarpa*, *Ambrosia chamissonis*).

[18] Dr. T. J. MABRY has reported (Second Natural Products Symposium, Mona, Jamaica, January 3–5, 1968) that parthenolide also occurs in some populations of *Ambrosia confertiflora* DC.

[19] Constunolide has recently been found in *Hymenoclea monogyra* (F. P. TORIBIO and T. A. GEISSMAN, to be published).

occur only in these two tribes in the Compositae.[20] A further finding bearing on this point is the discovery of a new lactone in both *Artemisa nova* and *Ambrosia acanthicarpa*.[21]

EXPERIMENTAL

Melting points were taken in capillaries and are corrected. I.r. spectra are for $CHCl_3$ solutions unless otherwise noted, and were measured on a Perkin–Elmer Model 21 spectrophotometer. NMR spectra were measured in $CDCl_3$ with tetramethylsilane as internal standard, with the use of a Varian A-60 instrument. TLC was carried out with Merck silica gel G; the developing solvent was in most cases $CHCl_3$-methanol, 4:1. In Table 1 are summarized the NMR data.

The specimens of *Ambrosia dumosa* used in this work were pooled specimens of a population of plants collected in a single restricted locality near Cabazon, California in March, 1967.[22] It is worth noting, however, that a previous collection of a polyploid plant made in a locality quite distant from this (Mecca, California) in 1966 showed, by TLC, substantially the same components as those seen in the polyploid material collected in 1967.

Extraction of Ambrosia dumosa *Gray. (polyploid)*

Leaves and flowering heads were stripped from the woody stems, dried at 40°, and ground in a Wiley mill. A sample of 2·15 kg of the polyploid material was extracted with chloroform at room temperature and the solvent removed under reduced pressure. The tarry residue was processed in the usual manner[23] to give a final residue of 54·1 g of a brown-yellow oily mixture that showed about ten components on TLC.

Isolation of Parthenolide (IV), Psilostachyin C (V), Burrodin (VI) and Apoludin (XII)

The crude oily extract (54·1 g) was chromatographed on silica gel (4·5 × 50 cm) with chloroform (2000 ml) and methanol (1000 ml) as the eluant; fractions of 50 ml were collected. Fractions 2–8 contained parthenolide and psilostachyin C; fractions 9–40 contained burrodin; and fractions 41–52 contained apoludin.

Parthenolide (IV)

Rechromatography of fr. 2–8 yielded 0·41 g (0·02 per cent) of parthenolide. It had m.p. 113–114°, and $[\alpha]_D^{27}$ −69° (*c*, 1·67, $CHCl_3$). (Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12. Found: C, 72·64; H, 8·22 per cent). Hydrogenation of 0·20 g of parthenolide (in ethyl acetate over 10 per cent Pd-charcoal) at atmospheric pressure afforded a quantitative yield of dihydroparthenolide, m.p. 134–135°. (Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86. Found: C, 71·97; H, 8·86 per cent). The dihydroparthenolide did not depress the m.p. of an authentic specimen;[24] the two materials showed identical TLC behavior and i.r. and NMR spectra.

Psilostachyin C (V)

Following the elution of parthenolide, psilostachyin C was obtained in later fractions. It had m.p. 227–229°, $[\alpha]_D^{29}$ −86·5° (*c*, 1·05, $CHCl_3$). The pure material did not depress the melting point of authentic psilostachyin C, and the two specimens showed identical TLC behavior and i.r. and NMR spectra. (Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63. Found: C, 68·05; H, 7·62 per cent).

Burrodin (VI)

Evaporation of fractions 9–40 yielded a residue that crystallized from ethyl acetate to give 6·57 g (0·31 per cent) of burrodin. It had m.p. 167–168°; $[\alpha]_D^{26}$ +108·5° (*c*, 0·4, $CHCl_3$). The u.v. spectrum showed λ_{max} 212 nm (ϵ, 10,800) and 285 nm (ϵ, 40); i.r. peaks were observed at 1760, 1750–1745 cm^{-1} (in $CHCl_3$); 3525, 3090, 1745 cm^{-1} (in KBr). Rotatory dispersion (in dioxan): $(\alpha)_{589}$ +40; $(\alpha)_{400}$ +350; $(\alpha)_{320}$ +1640; $(\alpha)_{315}$ +1460; $(\alpha)_{310}$ +1160; $(\alpha)_{305}$ +680; and $(\alpha)_{300}$ +200. Burrodin gave a positive Zimmermann test. (Calc. for: $C_{15}H_{20}O_4$: C, 68·16; H, 7·63. Found: C, 68·04; H, 7·43 per cent).

[20] Parthenolide has also been found in the family Magnoliaceae,[7] one of the rare occurrences of lactones of this group outside of the Compositae.

[21] Unpublished results, with M. A. IRWIN and T. G. WADDELL, to be reported in a paper in preparation. It is of interest that this compound (called artenovin) is a guaianolide, while the majority of the Ambrosieae lactones are pseudoguaianolides.

[22] Voucher specimens are preserved in the U.C.L.A. Herbarium: polyploid, No. 32067-ADUP; diploid: No. 32067-ADUD.

[23] T. A. GEISSMAN and F. P. TORIBIO, *Phytochem.* **6**, 1563 (1967).

[24] We are grateful to Dr. B. S. Joshi for a specimen of dihydroparthenolide and for a copy of the NMR spectrum of parthenolide.

TABLE 1. NMR PEAKS OF BURRODIN AND APOLUDIN DERIVATIVES

Compound	H_2	H_3	H_4	H_6	H_8	H_{13}	C_5-Me	C_{10}-Me	C_{11}-Me	Misc.
VI	4·5∼4·9br[a]				4·5∼4·9br[a]	5·71d (2·5) 6·33d (2·5)	1·05S	1·22d (7)		
VIA[e]	5·40br				4·60br	5·68d (2·5) 6·28d (2·5)	1·09S	1·15 (6)		2·08S[b]
VII	4·6–4·8br[a]				4·6–4·8br[a]		1·05S	1·06d (6)	1·20d (7·5)	
VIII	7·57dd (7·2)	6·13dd (7·3)			4·60∼4·97[c]	5·67d (2·5) 6·28d (2·5)	1·26S	1·15d (7)		
VIIIA[f]			4·4∼4·8br[a]		4·4∼4·8br[a]		0·93S	1·03d (8)	1·12d (9)	
XI					4·3∼4·8br		0·98S	1·00d (9)	1·12d (7)	
XII	4·37br		3·95t (8)	4·39d (9·5)		5·52d (3) 6·24d (3)	1·10S	1·36d (7)		
XIII	5·2∼5·3br		5·07 (9)	4·45d (9·0)		5·48d (3·5) 6·17d (3·5)	1·15S	1·24d (7)		2·08S[b, d]
XIV	4·32br		3·98t (8·5)	4·54S			0·87S	1·22d (7)	1·78S	
XV			4·35t (9)	4·82d (9·5)		5·62d (3) 6·22d (3)	0·95S	1·00d (7·5)		
XVI		6·08d (6)	7·75d (6)	5·08d (9·0)		5·66d (3) 6·38d (3)	1·18S	1·10d (7)		

All signals in the first five columns correspond to one proton, and all signals in the last four columns to three protons, unless otherwise specified. Multiplets are described as follows: S, singlet; d, doublet; dd, doublet of doublets; t, triplet; br, somewhat broadened ill-defined signals. Numbers in parentheses denote coupling constants in cps.

[a] Two protons; [b] acetate; [c] 4·60, 4·66, 4·74, 4·78, 4·85, 4·92 and 4·97 (total one proton); [d] six protons; [e] acetate of VI; [f] hexahydroanhydroburrodin.

Apoludin (XIII)

Evaporation of fractions 41–52 gave a residue that crystallized from ethyl acetate as colorless leaflets, m.p. 134–135°; $[\alpha]_D^{29}$ −42·5° (*c*, 0·4, $CHCl_3$). The u.v. spectrum showed λ_{max} 213 nm (ϵ, 10,230); i.r. peaks were observed at 1760, 1665 cm^{-1} ($CHCl_3$); 3420, 3080, 1740, 1650 cm^{-1} (KBr). (Calc. for $C_{15}H_{22}O_4$: C, 67·65; H, 8·33. Found: C, 67·50; H, 8·43 per cent).

Extraction of A. dumosa *Gray (diploid)*

Extraction of 350 g of leaves and flowering heads of the diploid form of *A. dumosa* (collected at the same time and place as the polyploid form) yielded 30·9 g of crude, brown-yellow oily residue. Chromatography of this material on silica gel (4·5 × 45 cm) was carried out with the use, successively, of 1000 ml of $CHCl_3$, 500 ml of 10 per cent acetone in $CHCl_3$, 500 ml of 15 per cent acetone in $CHCl_3$, 500 ml of 20 per cent acetone in $CHCl_3$, and methanol. Fractions of 50 ml were taken.

Fractions 1 and 2 yielded 50 mg (0·023 per cent) of parthenolide, identified by comparison with the authentic compound.

Fractions 6–14 yielded 1·77 g (0·5 per cent) of psilostachyin (III). Recrystallized from acetone, it formed colorless prisms, m.p. 215–217°; $[\alpha]_D^{29}$ −120° (*c*, 0·73, $CHCl_3$). It was identical (TLC, i.r., NMR mixed m.p.) with an authentic specimen. (Calc. for $C_{15}H_{20}O_5$: C, 64·27; H, 7·19. Found: C, 64·32; H, 7·37 per cent).

Fractions 15–28 yielded coronopilin (I), m.p. 180–181°; $[\alpha]_D^{29}$ −2·7° (*c*, 2·53, $CHCl_3$), identified by comparison with an authentic specimen. (Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63. Found: C, 68·28; H, 7·72 per cent).

Fractions 15–28 also contained ambrosiol (II), which was separated from coronopilin by rechromatography on silica gel. The purified compound formed colorless prisms from ethyl acetate, m.p. 120–122°; $[\alpha]_D^{29}$ −108° (*c*, 0·62, $CHCl_3$). It was identified by its spectral properties and by comparison (mixed m.p., NMR, TLC) with an authentic specimen. (Calc. for $C_{15}H_{22}O_4$: C, 67·65; H, 8·33. Found: C, 67·86; H, 8·30 per cent).

Burrodin Acetate

A solution of 150 mg of burrodin in 2 ml of pyridine and 2 ml of acetic anhydride was kept at room temperature and poured into ice water. The precipitate was collected by filtration and purified by passing its $CHCl_3$ solution through a column of silica gel. The product recovered from the eluate crystallized from ether as colorless leaflets, m.p. 116–117°; $[\alpha]_D^{30}$ +139° (*c*, 1·9, $CHCl_3$). It had λ_{max} 212 nm (ϵ, 12,000); and i.r. peaks at 1760, 1745, 1740 and 1660 cm^{-1}. (Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24. Found: C, 66·87; H, 7·49 per cent).

Dihydroburrodin (VII)

A solution of 600 mg of burrodin in 50 ml of ethyl acetate was hydrogenated at atmospheric pressure in the presence of 100 mg of Pt_2O. The reduction was interrupted after the absorption of one mole of H_2, and the solution filtered and evaporated to give the theoretical amount of dihydroburrodin. The compound formed colorless needles, m.p. 197–198°; $[\alpha]_D^{29}$ +141° (*c*, 0·33, $CHCl_3$). It showed λ_{max} 285 nm (ϵ, 45) and an ϵ value of 1288 at 217 nm. I.r. absorption was observed at 1770 and 1750 cm^{-1}. (Calc. for $C_{15}H_{22}O_4$: C, 67·65; H, 8·33. Found: C, 67·56; H, 8·35 per cent).

Anhydroburrodin (VIII)

To a solution of 500 mg of burrodin in 2 ml of pyridine was added 0·3 ml of methanesulfonyl chloride, and after 30 min at ice-bath temperature water was added and the mixture extracted with $CHCl_3$. The product, isolated from the chloroform solution after passage through a column of silica gel, formed colorless needles from ether (prisms from ethyl acetate). It had m.p. 125–126°; $[\alpha]_D^{30}$ +37·8° (*c*, 2·5, $CHCl_3$). It had λ_{max} 217 nm (ϵ, 14,500) and 320 nm (ϵ, 50), and i.r. absorption at 1770, 1720, 1660 and 1580 cm^{-1}. Rotatory dispersion (in dioxan): $(\alpha)_{420}$ −40; $(\alpha)_{400}$ −80; $(\alpha)_{367}$ −400; $(\alpha)_{297}$ +800. (Calc. for $C_{15}H_{18}O_3$: C, 73·15; H, 7·37. Found: C, 73·25; H, 7·59 per cent).

Tetrahydroanhydroburrodin (XI)

A solution of 40 mg of anhydroburrodin in 30 ml of ethyl acetate containing 5 mg of 10 per cent Pd–C was hydrogenated at ordinary pressure until hydrogen uptake ceased. The product (35 mg), isolated in the usual way, formed colorless prisms from ether, m.p. 146–147°; $[\alpha]_D^{28}$ +121° (*c*, 0·48, $CHCl_3$). The i.r. spectrum showed peaks at 1768 and 1738 cm^{-1}. (Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86. Found: C, 72·08; H, 8·55 per cent).

Hexahydroanhydroburrodin

Hydrogenation of 520 mg of anhydroburrodin in acetic acid in the presence of Pt_2O was continued until 3 moles of hydrogen had been taken up. The product, isolated in the usual way, formed colorless leaflets

(254 mg) from ether; m.p. 139–141°; $[\alpha]_D^{28}$ +46·5° (*c*, 0·7, $CHCl_3$); i.r. peaks at 3400, 1770 cm^{-1}. (Calc. for $C_{15}H_{24}O_3$: C, 71·39; H, 9·59. Found: C, 71·53; H, 9·32 per cent).

Apoludin Acetate (*XIII*)

Acetylation of 200 mg of apoludin with 3 ml of acetic anhydride and 3 ml of pyridine at room temperature yielded 259 mg of apoludin acetate, colorless leaflets from ether, m.p. 107–108°; $[\alpha]_D^{26}$ −54·3° (*c*, 0·7, $CHCl_3$). The u.v. spectrum showed λ_{max} 217 nm (ϵ, 13,700), and i.r. peaks were observed at 1760, 1745, 1725 and 1665 cm^{-1}. (Calc. for $C_{19}H_{26}O_6$: C, 65·13; H, 7·48. Found: C, 65·07; H, 7·64 per cent).

Isoapoludin (*XIV*)

Hydrogenation of 1·0 g of apoludin in ethyl acetate in the presence of 100 mg of platinum oxide was continued for 6 hr, after which there was isolated 310 mg (after purification over a column of silica gel) of a product that crystallized from ethyl acetate as colorless prisms, m.p. 149–152°; $[\alpha]_D^{29}$ +32° (*c*, 0·73, $CHCl_3$). It had λ_{max} 221 nm (ϵ, 7800) and i.r. peaks at 1755 and 1660 cm^{-1}. (Calc. for $C_{15}H_{22}O_4$: C, 67·65; H, 8·33. Found: C, 67·60; H, 8·12 per cent).

2-*Dehydroapoludin* (*XV*)

To a solution of 300 mg of apoludin in 80 ml of acetic acid was added 600 mg of CrO_3. The solution was kept for 2 hr at room temperature, poured into water, and the mixture extracted with $CHCl_3$. The product, purified by chromatography in $CHCl_3$ solution on a silica column, formed colorless needles from ethyl acetate-ether, m.p. 157–160° (dec.). It had λ_{max} 215 nm (ϵ, 10,600) and 285 nm (ϵ, 300), and i.r. peaks at 1775, 1750 and 1660 cm^{-1}. A Zimmermann test was positive. (Calc. for $C_{15}H_{20}O_4 \cdot H_2O$: C, 63·81; H, 7·85. Found: C, 63·62; H, 7·66%).

Anhydrodehydroapoludin (*XVI*)

A solution of 400 mg of 2-dehydroapoludin in 4 ml of pyridine and 0·4 ml of methanesulfonyl chloride was kept in an ice bath for 2 hr and poured into water. The product, isolated by $CHCl_3$ extraction and purified by chromatography over silica gel, formed colorless prisms from ether, m.p. 190–191°; $[\alpha]_D^{29}$ +64° (*c*, 0·5, $CHCl_3$). It had λ_{max} 218 nm (ϵ 18160) and 320 nm (ϵ, 85), and showed i.r. absorption at 1766, 1716, 1660 and 1600 cm^{-1}. (Calc. for $C_{15}H_{18}O_5$: C, 73·15; H, 7·37. Found: C, 73·18; H, 7·39 per cent).

Apoludin Sulfite (*XVII*)

To an ice-cooled solution of 80 mg of apoludin in 3 ml of pyridine was added dropwise 0·5 ml of thionyl chloride. After one hour at 0°, the solution was poured into water and mixture extracted with $CHCl_3$. The $CHCl_3$ layer was washed with dil HCl and water, dried (Na_2SO_4), and passed through a column of silica gel. The product (58 mg) formed colorless prisms from ethyl acetate and had m.p. 115–117° (dec.). It showed i.r. absorption at 1775, 1660 and 1020 cm^{-1}. (Calc. for $C_{15}H_{20}O_2S$: C, 57·67; H, 6·45. Found: C, 57·90; H, 6·51 per cent).

Determination of Configuration (*A*) *at C-4 in 2-Dehydroapoludin and* (*B*) *at C-2 in Burrodin*

A. A solution of 320 mg (1·2 mmole) of 2-dehydroapoludin and 780 mg of racemic 2-phenylbutanoic anhydride in 3 ml of pyridine was kept at room temperature for 2 days. After working up the reaction mixture by the usual procedure[14,5] there was recovered 457 mg of 2-phenylbutanoic acid with $[\alpha]_D^{29}$ +7·7°. The identity of the recovered acid was substantiated by comparison (NMR and i.r. spectra) with authentic material.

B. Treatment of 264 mg (1 mmole) of burrodin with 621 mg of 2-phenylbutanoic anhydride in the manner described in *A* resulted in the isolation of 350 mg of 2-phenylbutanoic acid with $[\alpha]_D^{28}$ −3·4°.

Acknowledgement—The authors are grateful for support provided under U.S.P.H.S. Research Grant GM-14240-01/02; to Professors W. W. Payne and P. H. Raven for valuable comment and identification of plant material; to Miss Judith Levy for the identification of psilostachyin in a specimen of *A. dumosa* (diploid) collected from a locality near Yuma, Arizona; to Dr. T. J. Mabry for several reference specimens; and to Miss Heather King for microanalyses.