was chromatographed preparatively on thin layer chromatographic plates (silica gel) using 9:1 cyclohexane–diethylamine as the solvent.

This method of oxidation and rearrangement of voacangine was repeated with an additional 0.040 g of voacangine. The bands on the thin layer chromatograms corresponding in R_f value to rupicoline were removed from the plates and eluted with methanol. The base derived in this manner was converted to a hydrochloride salt and recrystallized from isopropyl alcohol. The total yield was approximately 2 mg of fine yellow needles.

After a second recrystallization of the infrared spectrum of this material was taken in a potassium bromide pellet. This spectrum is reproduced in Figure 1b, superimposed on the spectrum of rupicoline.

The X-ray powder method was also used to prove the identity of this compound with rupicoline hydrochloride. A sample of each of the compounds was powdered and placed in a capillary tube. X-Ray exposures of 20 and 122 hr were then made using the Straumanis technique. Chromium radiation with a vanadic acid filter was used (the λ_{max} of Cr Kα = 2.2909 A). The camera used had a diameter of 99.812 mm. Arcs were measured on a comparator to ±0.02 mm. Relative intensities of the lines were estimated visually. The X-ray powder spectra were identical.

Further Oxidation of Voacangine.—A small sample of voacangine (0.010 g) was treated in the exact manner described earlier for the isolation of the ether extractable organic bases from the plant. The resulting fraction was thin layer chromatographed with rupicoline as a comparison. No spot corresponding to rupicoline could be detected in the material treated in this way.

A sample of voacangine (0.010 g) was refluxed in ether for approximately 1 month and the ether was evaporated to dryness. Thin layer chromatography of a sample of the residue did not reveal a spot of R_f value corresponding to rupicoline. The residue was then allowed to stand for 2 weeks in the sunlight and another sample was taken and chromatographed. A faint spot corresponding to rupicoline in R_f value and color could then be detected. Most of the material was unreacted voacangine.

Acknowledgments.—The authors wish to express their appreciation to Dr. R. F. Raffauf of Smith, Kline and French Laboratories for obtaining the plant materials studied, to Danual Kuwada of the Jet Propulsion Laboratory, and H. Budzikiewicz of Stanford University for obtaining the mass spectrum of rupicoline.

Chamissonin, a Germacranolide from an *Ambrosia* Species

T. A. GEISSMAN, R. J. TURLEY, AND S. MURAYAMA

Contribution No. 1911 from the Department of Chemistry, University of California, Los Angeles, California

Received January 24, 1966

Chamissonin, a sesquiterpenoid lactone present in *Ambrosia chamissonis* (Less.) Greene, is shown to be a germacranolide, a structural type known in other tribes of the *Compositae* but hitherto unobserved in members of the subtribe *Ambrosiinae* of the tribe *Heliantheae*.

Ambrosia chamissonis (Less.) Greene (*Franseria chamissonis* ssp. *bipinnatisecta* Less.) (*Compositae*, tribe *Heliantheae*, subtribe *Ambrosiinae*)[1,2] is a perennial common to coastal Southern California. In view of the occurrence of sesquiterpenoid lactones in numerous other members of the *Ambrosiinae*,[3-5] an examination of *A. Chamissonis* was undertaken.

The plant is rich in sesquiterpenoid lactonic material, and the crude syrup that was obtained in *ca.* 1% yield (see Experimental Section) crystallized spontaneously to afford the compound chamissonin. Although crude chamissonin was obtained with ease, it proved to be unstable and appeared to polymerize when attempts were made to purify it. The pure compound was obtained by slow crystallization from benzene and, when free of contaminants, could be recrystallized from benzene. The mother liquors from which chamissonin separated, and which contained much of the compound, could be chromatographed on silica gel to yield eluate fractions that showed only a single (chamissonin) spot on thin layer chromatograms but which yielded the crystalline compound only reluctantly and in poor yield; yet these same syrupy materials gave high yields of the diacetate.

Chamissonin, mp 124–125°, has the composition $C_{15}H_{20}O_4$. It shows ultraviolet absorption at 200 mμ (ϵ 12,600), and its infrared spectrum contains the prominent peak at 1765 cm^{-1} and the less intense peak at 1650 cm^{-1} that are characteristic of the α-methylene γ-lactone, a structural feature common to many sesquiterpenoid lactones of the *Compositae* and especially prevalent in those of the *Ambrosiinae*. Except for an intense absorption in the hydroxyl region (3500 cm^{-1}), no other conspicuous structural features were obvious from the infrared spectrum. The nmr spectrum of chamissonin (in pyridine) showed two methyl groups (3 H singlets) at 1.67 and 2.26 ppm, but was not as clearly interpretable as the spectra of the derivatives to be discussed below.

Chamissonin readily formed a diacetate and a dibenzoate; both of these had compositions that agreed with the formulation of chamissonin as a dihydroxy lactone, $C_{15}H_{20}O_4$. The diacetate showed a Kuhn–Roth terminal methyl number of 3.3 and its infrared spectrum, which lacked absorption in the hydroxyl region, showed peaks at 1735 cm^{-1} (acetate) and 1755 and 1645 cm^{-1} (lactone). The nmr spectrum of the diacetate showed the acetyl methyl groups (as 3 H singlets) at 2.12 and 2.07 ppm, two methyl groups (3 H singlets) at 1.80 and 1.73 ppm, and the characteristic pair of doublets (J = 2 cps) for protons of the exocyclic methylene group at 6.27 and 5.94 ppm. The region of *ca.* 2–6 ppm is a complex grouping of signals which includes a 1 H multiplet centered at 4.3 ppm, assignable to the CH—O methine proton of the lactone grouping. The high-field position of this signal, and its multiplicity, indicate that it is nonallylic, and is in the structural grouping –CH–*CH*–CH–. The region between 2.1 and *ca.* 3.4 ppm integrates for five protons, and includes protons that are not adjacent to oxygenated functions. A group of four protons that give rise to a broad complex between 4.8 and 5.5 ppm includes the two methine

(1) The inclusion of the genus *Franseria* in *Ambrosia* is proposed by W. W. Payne [*J. Arnold Arboretum*, **45**, 401 (1964)].

(2) P. A. Munz and D. D. Keck, Ed., "A California Flora," University of California Press, Berkeley, Calif., 1959.

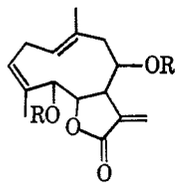
(3) W. Herz and Y. Sumi, *J. Org. Chem.*, **29**, 3438 (1964).

(4) W. Herz and G. Hogenauer, *ibid.*, **26**, 5011 (1961).

(5) T. A. Geissman and R. J. Turley, *ibid.*, **29**, 2553 (1964).

protons of two secondary acetoxy groupings and two vinyl protons.

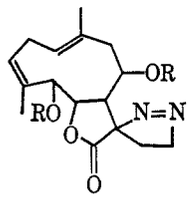
These spectroscopic observations can be accommodated in the expressions I for chamissonin and II for its diacetate.



I, R=H
II, R=Ac
III, R=COC_6H_5

Chamissonin dibenzoate (III) had infrared absorption peaks at 1760 and 1705 cm^{-1} (lactone and benzoyl). The nmr spectrum reveals the structural features shown in structure III, and, because of the unequivocal proton integration made possible by the ten (six at 7.56 and four at 8.08 ppm) aromatic protons, the complex of protons in the range 4.9–5.8 ppm is seen clearly to be four. Two of these can be the methine protons of the benzoyloxy groups and two the vinylic protons at C-1 and C-3.

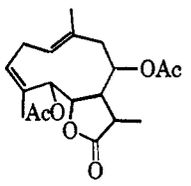
The chemical behavior of chamissonin confirmed the conclusion that it is a germacranolide of the indicated structure (I). Treatment of chamissonin with diazomethane in ether yielded the crystalline monopyrazoline (IV). The corresponding pyrazoline (V) was obtained



IV, R=H
V, R=Ac

by similar treatment of the diacetate. The nmr spectrum of V shows the two methyl group singlets at 1.54 and 1.77 ppm and the absence of the signals for the exocyclic methylene group while the remainder of the skeletal hydrogen atoms are substantially as observed in the spectra of II and III. The most informative feature of the nmr spectrum of V is the marked upfield shift of one of the acetyl methyl group signals. In the nmr spectrum of II the two acetyl methyl groups appear as singlets at 2.07 and 2.12 ppm. In the spectrum of V, one of these signals is still seen at 2.07 ppm, but the other is now found at 1.82 ppm. This shift of 0.3 ppm indicates that one of the acetyl groups is strongly affected by the formation of the pyrazoline ring, and that its position in the molecule is such that it is strongly shielded by the azo linkage in V. The placing of one of the acetoxyl groups at C-8 can account for this; and it should be noted that this position is the one most commonly hydroxylated in lactones of the type to which chamissonin belongs. *E.g.*, hydroxybalchanolide and hydroxycostunolide, both found in *Artemisia balchanorum*,[6] have the dispositions of the lactone ring and the C-8 hydroxyl group shown in I. Additional confirmation of the presence of the exocyclic methylene group on the lactone ring was obtained by ozonolysis of chamissonin, with the formation of formaldehyde in 27% yield.

Attempts to hydrogenate chamissonin led in most experiments to unsatisfactory results, but, when the uptake of hydrogen was interrupted after the absorption of 1 mole and the product acetylated, there was obtained a crystalline dihydrochamissonin diacetate. The lactone band appeared at 1765 cm^{-1} in the infrared spectrum, and the nmr spectrum revealed the absence of the low-field signals of the exocyclic methylene group and the appearance of a new three-proton doublet at 1.3 ppm. It is clear that in the dihydro compound it is the exocyclic methylene group that has been reduced (VI). Signals for the other protons in the molecule are essentially unchanged from II.



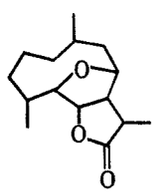
VI

When chamissonin was hydrogenated in ethanol solution with the use of an equal weight of 10% palladium–charcoal, the absorption of hydrogen proceeded until between 3 and 4 moles (in various experiments) had been absorbed. The product from this hydrogenation was a noncrystallizable mixture of at least three components (by thin layer chromatography). Chromatographic separation on an alumina column afforded a 23% yield of a crystalline compound (A), which was eluted with carbon tetrachloride and recrystallized from hexane. After removal of A from the column, elution with chloroform–carbon tetrachloride yielded a second material in *ca.* 60% yield (based on the total crude hydrogenation product). This material (B) could not be crystallized. Its behavior on thin layer chromatography indicated that it was less polar than chamissonin but more so than compound A. Its infrared spectrum showed the presence of a hydroxyl group (3580 cm^{-1}) and the (saturated) γ-lactone (1780 cm^{-1}), but attempts to prepare a crystalline acetate or benzoate were not successful. At length, the compound B was oxidized with chromic acid in acetone, with the formation of a crystalline compound (C) isomeric with A ($C_{15}H_{24}O_3$). Compound C showed no hydroxyl group absorption in the infrared but its spectrum contained, in addition to the lactone peak at 1780 cm^{-1}, a carbonyl band at 1690 cm^{-1}. That the compound was indeed a ketone was demonstrated by the preparation of a yellow 2,4-dinitrophenyl hydrazone.

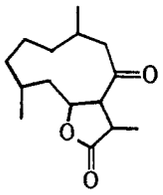
The nmr spectra of compounds A and C are in full accord with their formulation as the ether VII and the ketone VIII.

The three methyl groups of VII are clearly seen among a complex of signals that appear in the region between 1.0 and 2.2 ppm, and only three protons are found at lower fields. One of these is in a sharp one-proton singlet at 3.25 ppm, and the other two are found in a 2 H multiplet centered at *ca.* 8.2 ppm. The infrared spectrum of VII shows only a single peak between the

(6) H. Krasch, V. Herout, M. Suchý, and F. Šorm, *Collection Czech. Czech. Chem. Commun.*, **26**, 2612 (1961).



VII



VIII

C–H signals at *ca.* 2900 cm^{-1} and the beginning of the fingerprint region at 1450 cm^{-1}; this well-defined peak, at 1773 cm^{1}, is clearly that of the saturated γ-lactone, and consequently the third oxygen atom must be present in an ether linkage. The two-proton signal at 4.2 ppm in the nmr spectrum of VII must include the methine proton of the lactone grouping, and thus there are two protons, that at 3.25 ppm and one of the two in the 4.2 ppm multiplet, to be accounted for. From the composition of VII it is clear that the compound is a cyclic ether and monocarboxyclic, and that chamissonin is a germacranolide.

The singlet character of the high-field signal at 3.25 ppm is unusual, but its position is consistent with that of an α proton on a five- or six-membered cyclic ether.[7] The α protons on four- or three-membered cyclic ethers are found at chemical shifts different from this.[7,8]

The high-field location of the secondary methyl groups of VII indicates that these are not present in CH_3—C—O linkages and that the ether bridge is not between positions 4 and 9. Moreover, were the carbon skeleton of chamissonin already of the pseudoguaianolide type[9] (with a methyl group at C-5 instead of C-4), with the ether ring junction in VII at C-4, this would require a structure for chamissonin to which certain additional observations, discussed below, could not be accommodated. Compound VII is thus formulated with the ether linkage in the position shown.

The ketone, compound C (VIII), gives an nmr spectrum that is in full accord with these interpretations. This compound shows one proton (1 H, multiplet) at 4.95 ppm, and no other proton signals below 3.0 ppm. A group of four protons between *ca.* 2.2 and 3.0 ppm clearly corresponds to the four protons adjacent to the two carbonyl groups in VIII, while the complex of the remaining protons between 1.0 and 2.2 ppm represents the numerous alicyclic methylene and methyl groups of the molecule. Within this group there are discernable the three doublets of the three secondary methyl groups. One of these, the methyl group of the lactone ring, gives rise to the lower field signal at 1.25 ppm (J = 6 cps); the other two appear at 0.96 and 1.04 ppm (J = 5 cps, both). The high-field position of these latter two signals indicates that they are not adjacent to the carbonyl group, a conclusion that provides an additional basis for the assignment to the ketone of the structure (VIII) shown.

The ketone (VIII) is isomeric but not identical with the compound of the same gross structure formed from isobalchanolide,[6] from which it probably differs in one or more of the five possible centers of asymmetry.

(7) L. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Ltd., London, 1959, pp 55–85.

(8) N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962, No. 33.

(9) This rearranged skeleton is characteristics of the 5/7 bicyclic compounds of the *Ambrosiinae*.

A variety of modifications of the hydrogenation of chamissonin and its diacetate were studied in an attempt to saturate the double bonds without bringing about other alterations in the functionality. Probably because of the allylic character of the C-5 hydroxyl group, which is removed in the course of formation of the ketone VIII, most hydrogenation experiments yielded complex and intractable mixtures from which no crystalline products could be obtained. Except for dihydrochamissonin acetate, and the compounds VII and VIII, no useful products could be obtained.

An additional confirmation of the presence of two double bonds (in addition to that in the lactone grouping) was obtained by quantitative bromination. Chamissonin absorbs bromine instantly and titration to a persistent bromine color could be carried out with reproducible results. Chamissonin required 2.0 moles of bromine/mole (the double bond of the lactone ring of coronopilin[3,4] is resistant to the addition of bromine) and dihydrochamissonin required 1.9 moles.

This high degree of unsaturation was reflected in the extreme reactivity of chamissonin to oxidation under a variety of conditions. No characterizable products could be obtained with the use of manganese dioxide, *t*-butyl chromate in pyridine, the Oppenauer reagent under mild conditions, or N-bromosuccinimide. In an experiment in which aqueous chromic acid (sufficient to oxidize one hydroxyl group) was added in repeated small portions to an ice-cooled solution of chamissonin in acetone, with periodic examination of the products in the ultraviolet, no new or enhanced absorption appeared in the range expected for an α,β-unsaturated ketone. Thin layer chromatography of the reaction mixture at each stage of the oxidation showed only materials that were much less mobile than chamissonin, an indication that oxidation was proceeding to the formation of complex materials. The anticipated 3-ene-5-one would be expected to have an R_f value higher than that of chamissonin.

The placing of the two double bonds of the cyclodecadienolide ring system as shown in structure I finds support in the following considerations. All of the nmr evidence requires that the two methyl groups be CH_3—C=C in type, and thus that the double bonds be located at the (1,10/4,5), (1,10/3,4), (9,10/4,5), or (9,10/3,4) positions. Chamissonin diacetate (II) and chamissonin dibenzoate (III) and the pyrazoline derivative of chamissonin (IV) all show a well-separated two-proton signal at 2.92 ppm (IV), 3.10 ppm (II), and 3.35 ppm (III). The spectra of balchanolide[6] and 12-methoxydihydrocostunolide[10] show no comparable proton absorption below 2.5 ppm, a region above which the singly allylic methylene protons are to be expected (and in which the C-7 and C-9 protons of chamisson are indeed observed). The 2-H doublet (most clearly seen in III) at 3.45–3.26 (centered at 3.25) ppm can best be accounted for by structures I, etc., in which the methylene group at C-2 is flanked by the 1,10, and 3,4 double bonds. In a 9,10/4,5-diene the methylene protons at C-2 would be flanked by methylene groups, and thus would appear at fields higher than those at which any protons appear, and in the 1,10/4,5 and 9,10/3,4 dienes it would be difficult to account for the

(10) G. H. Kulkarni, A. Paul, A. S. Rao, G. R. Kelkar, and S. C. Bhattacharya, *Tetrahedron*, **12**, 178 (1961).

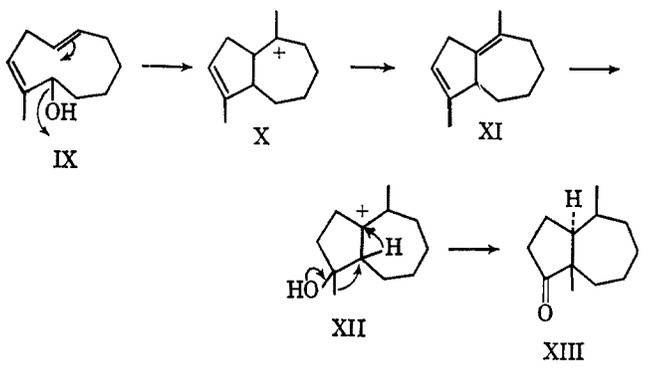
special character and the low-field position of the two-proton group in the 3-ppm region of the spectrum.

The stereochemistry of chamissonin remains to be established in detail. It is pertinent to note that, while in the nmr spectrum of chamissonin diacetate the region in which the two methyl groups (3-14 and C-15) are found contains only the six protons of these groups, in chamissonin dibenzoate the same region shows seven protons, while the 2–3-ppm region, which in the diacetate shows five protons, contains but four in the dibenzoate. It appears that the benzoyl group at C-8 effects a shielding of the C-7 proton, moving it to higher field, and thus that the C-7 proton is *cis* to the benzoyloxy group. If the C-7/C-11 bond of the lactone ring is β oriented, as is usual in compounds of this class, this would indicate that the C-8 hydroxyl group of chamissonin is α disposed, as are the corresponding hydroxyl groups of the known germacranolides balchanolide,[6] hydroxycostunolide,[11] scabiolide,[12] cnicin,[12] and arctiopicrin.[13]

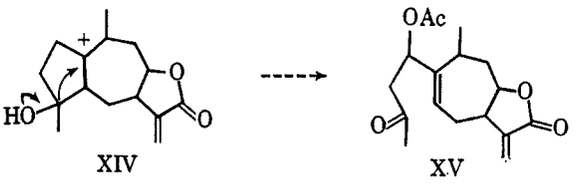
Further studies to clarify the stereochemistry of chamissonin are in progress.

It is of interest to note that *Ambrosia* species are characterized by the presence of pseudoguaianolides typified by parthenin,[14] coronopilin,[4,5] ambrosin,[3] damsin,[3] and several other compounds derived from the fundamental 5,10-dimethylguaianolide nucleus. Chamissonin represents what appears to be an uncyclized precursor of these types, but also appears to represent a stage in their biosynthesis that is earlier than that at which the migration of the methyl group occurs. The following scheme shows a plausible sequence of events in the biosynthetic pathway (the lactone and C-8 hydroxyl groups are not directly relevant to the stages illustrated and are omitted from the formulation) leading through the normal isoprenoid precursor (such as I) to the abnormal pseudoguaianolide system (XIII) found in the *Ambrosiinae* and the *Helenieae* (Chart I). It is interesting to note that the

CHART I



unusual sesquiterpene lactone xanthinin (XV),[15] a constituent of *Xanthium pennsylvanicum*, also a member of the *Ambrosiinae*, is readily derived from an intermediate (XIV) having the chief structural features of the conjectured intermediate XII.



While these structural relationships are suggestive, they need the support of additional information. The further examination of the still unknown constituents of *A. chamissonis* and of *Xanthium* may provide further evidence that will clarify the picture of the biosynthetic relationships in these plant groups.

Experimental Section

Melting points were taken in capillary tubes and are corrected. Nuclear magnetic resonance spectra were determined in deuteriochloroform except where specifically indicated otherwise, with tetramethylsilane as an internal standard, using a Varian Model A-60 spectrometer. Thin layer chromatography was carried out with the use of silica gel G; the solvent used in most cases was chloroform–methanol (9:1, v/v), and visualization was accomplished with iodine vapor or a spray of potassium permanganate. Ultraviolet spectra were determined in methanol solution and infrared spectra in chloroform.

Isolation of Chamissonin (I).—Air-dried, ground leaves and stems of *A. chamissonis* (Less.) Greene, collected along the beaches near Venice, Calif., were extracted with methylene chloride at room temperature. The combined extract from 1450 g of plant material was evaporated under reduced pressure to a dark, tarry residue which was taken up in 200 ml of ethanol. After the addition of 600 ml of hot water containing 10 ml of saturated lead acetate solution, the mixture was well shaken and allowed to stand until the tarry material had separated. The aqueous solution was decanted, the tar was reextracted again in the same way, and the combined aqueous extracts were clarified by filtration through celite. The clear yellow filtrate was extracted with six 50-ml portions of chloroform and the extract was dried and evaporated to an oily residue (35.3 g). The oil partly crystallized when triturated with a little chloroform. The solid was collected, and the oily filtrate was allowed to stand after the addition of 50 ml of benzene, when additional solid was separated. The yield of crude crystalline material was 23.8 g. When attempts were made to recrystallize this material from chloroform, ethyl acetate, or benzene the product tended to separate as an oil which crystallized reluctantly upon standing. It was at length found that when a solution in benzene was allowed to stand in the cold, chamissonin crystallized slowly over a period of time, and successive crops could be collected. This material could be recrystallized from benzene, from which it formed white leaflets: mp 124–125°. It had $[\alpha]^{25}D$ −19.8° (*c* 2.2, ethanol). It showed a single spot on a thin layer chromatogram (tlc). The mother liquors and washings showed several spots on tlc, including a prominent one of chamissonin. It is apparent that a part of the reason for the great difficulty in purifying crude chamissonin by crystallization is the presence of persistent impurities, for the acetylation of crude chamissonin was characterized by the formation of dark brown acetylation reaction mixtures, while purified chamissonin can be acetylated with the production of no more than a yellow color in the acetic anhydride–pyridine reaction mixture.

In another experiment, the benzene mother liquors from which chamissonin had separated were chromatographed on silica gel. A fraction eluted with benzene–chloroform showed essentially one spot (of chamissonin) on tlc. Even this crystallized reluctantly, but could be acetylated cleanly to give a good yield of pure chamissonin diacetate.

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.27; H, 7.82; Kuhn–Roth, 1.6 methyl groups.

Chamissonin Diacetate (II).—A solution of 1 g of chamissonin in a mixture of 1 ml of pyridine and 3 ml of acetic anhydride was warmed on the water bath for 15 min and poured into water. The mixture was extracted with ether and the ether extract was washed with water, aqueous sodium bicarbonate, and aqueous hydrochloric acid, dried, and evaporated. The residue was

(11) M. Suchý, V. Herout, and F. Šorm, *Collection Czech. Chem. Commun.*, **28**, 1618 (1963).
(12) M. Suchý, V. Herout, and F. Šorm, *ibid.*, **27**, 2398 (1962).
(13) M. Suchý, V. Herout, and F. Šorm, *ibid.*, **24**, 1542 (1959).
(14) W. Herz, H. Watanabe, M. Miyazaki, and Y. Kishida, *J. Am. Chem. Soc.*, **84**, 2601 (1962).
(15) P. G. Deuel and T. A. Geissman, *ibid.*, **79**, 3778 (1957).

dissolved in 5 ml of ethanol, from which it crystallized on cooling (1.1 g). The diacetate does not show the instability of chamissonin and can be recrystallized satisfactorily. The pure compound had mp 174–175°, $[\alpha]^{23}D$ −51.8° (*c* 2.08, chloroform). A Kuhn–Roth determination showed 3.3 methyl groups.

Anal. Calcd for $C_{19}H_{24}O_6$: C, 65.50; H, 6.94. Found: C, 65.59; H, 7.15.

Chamissonin Dibenzoate (III).—A mixture of 0.5 g of chamissonin, 1 ml of benzoyl chloride, and 3 ml of pyridine was allowed to stand at room temperature overnight, diluted with water, and the mixture was extracted with ether. The ether solution was washed with aqueous sodium carbonate, dilute hydrochloric acid, and water, dried, and evaporated. The residual material was passed through a column of alumina, from which elution with ether gave 0.26 g of a crystalline material. Purified by recrystallization from methanol, the compound had mp 169–173°.

Anal. Calcd for $C_{20}H_{28}O_6$: C, 73.71; H, 5.97. Found: C, 73.76; H, 6.02.

Monopyrazoline of Chamissonin (IV).—An ether solution of the diazomethane prepared from 1 g of nitrosomethylurea was added to a solution of 0.50 g of chamissonin in 50 ml of ether, and the solution allowed to stand overnight. After destruction of the excess diazomethane with acetic acid, the solution was concentrated, with the formation of a white solid. Recrystallization from chloroform gave the colorless pyrazoline: mp 168–169° dec.

Anal. Calcd for $C_{16}H_{22}N_2O_4$: C, 62.72; H, 7.24; N, 9.14. Found: C, 62.66; H, 7.17; N, 9.12.

Monopyrazoline of Chamissonin Diacetate (V).—A solution of 0.25 g of chamissonin diacetate in 50 ml of chloroform was treated with an ether solution of diazomethane (from 1 g of nitrosomethylurea). After 16 hr the excess diazomethane was decomposed with acetic acid and the solution evaporated. The residue (0.16 g) crystallized from carbon tetrachloride and the product was recrystallized from methanol–carbon tetrachloride. It had mp 143–144° dec.

Anal. Calcd for $C_{20}H_{26}N_2O_6$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.77; H, 6.51; N, 7.35.

Dihydrochamissonin Diacetate (VI).—To a presaturated sample of 0.26 g of 10% palladium–charcoal in 20 ml of ethanol was added a solution of 0.50 g of chamissonin in 5 ml of ethanol. Hydrogenation was allowed to proceed at 1 atm of pressure until 44.6 ml (1 mole) had been absorbed. The catalyst was removed and the solvent was evaporated to leave an amorphous residue which was passed through a column of silica gel (10 g) with 250 ml of chloroform–methanol. The recovered material was a colorless, glassy residue which could not be crystallized. This material was acetylated with acetic anhydride in pyridine to give the crystalline acetate; recrystallized from ethanol, it had mp 132–135°.

Anal. Calcd for $C_{19}H_{26}C_6$: C, 65.12; H, 7.48. Found: C, 65.24; H, 7.55.

Hydrogenation of Chamissonin. Compounds VII and VIII.—A suspension of 2.1 g of 10% palladium on charcoal in 30 ml of ethanol was saturated with hydrogen. A solution of 1.9 g of chamissonin in 8 ml of ethanol was added and hydrogenation was continued at atmospheric pressure until 525 ml of hydrogen had been absorbed. Hydrogenation ceased at this point, and in another 4 hr no more hydrogen was taken up. The filtered solution was evaporated to give a brown, glassy residue which showed three spots on a thin layer chromatogram. This material was chromatographed on 60 g of alumina (activity IV). Elution with 150 ml of carbon tetrachloride gave 0.36 g of crystalline material (A). This was recrystallized several times from hexane to give white needles: mp 150–152°; $[\alpha]^{26}D$ 81.4° (*c* 4.8, chloroform). The infrared spectrum showed the complete absence of absorption in the hydroxyl region and but one peak in the carbonyl region (1773 cm^{-1}, lactone).

Anal. Calcd for $C_{15}H_{24}O_3$(compound VII): C, 71.39; H, 9.59. Found: C, 71.46; H, 9.53.

Continued elution of the column with 200 ml of chloroform–carbon tetrachloride (1:1) yielded 0.92 g of a noncrystallizable glassy material. Its infrared spectrum showed the presence of hydroxyl group absorption and a lactone band at 1780 cm^{-1}.

This material was oxidized by dissolving it in 5 ml of acetone and adding in small portions a 2.67 *N* solution of chromic acid in dilute sulfuric acid until the red-brown color persisted. The addition of water produced a crystalline precipitate (0.12 g), which was recrystallized from carbon tetrachloride–hexane to give white needles: mp 151–152°; $[\alpha]^{26}D$ −35.8° (*c* 4.8, chloroform). The compound showed no hydroxyl absorption in the infrared, but showed two well resolved peaks in the carbonyl region (1780 and 1690 cm^{-1}). It showed no high-intensity absorption in the ultraviolet from 200 to 300 mμ.

Anal. Calcd for $C_{15}H_{24}O_3$ (compound VIII): C, 71.39; H, 9.59. Found: C, 71.42; H, 9.56.

2,4-Dinitrophenylhydrazone of VIII.—The 2,4-dinitrophenylhydrazone of the keto lactone was prepared in the usual way. Recrystallized from ethanol it formed yellow leaflets: mp 197–198°.

Anal. Calcd for $C_{21}H_{28}N_4O_6$: C, 58.32; H, 6.53; N, 12.96. Found: C, 58.44; H, 6.55; N, 12.75.

Quantitative Bromination of Chamissonin and Dihydrochamissonin.—A solution of 0.47 g of bromine in 10 ml of methanol was added from a microbaret, with stirring, to an ice-cooled solution of 0.136 g of chamissonin in 5 ml of methanol. The bromine color was discharged immediately until 1.77 ml (2.02 mole equiv) had been added, when the color persisted. Attempts to isolate a crystalline product were fruitless.

Using the same procedure, dihydrochamissonin was titrated with bromine, with the uptake of 1.9 mole equiv. Again, no characterizable product could be isolated.

Ozonolysis.—A solution of 0.11 g of chamissonin in 25 ml of methylene chloride and 1 ml of methanol was ozonized for 45 min at Dry Ice temperature, the exhaust gases being passed through a water trap. The dark blue methylene chloride solution was allowed to warm to room temperature, the contents of the water trap were added, and the mixture was refluxed for 20 min. After the addition of 8 g of magnesium sulfate the aqueous solution was distilled, and to the distillate (100 ml) was added 20 ml of a saturated aqueous solution of dimedone. The crystalline material that formed (32 mg, 27%) melted at 188–191° and showed no depression in melting point on admixture with authentic formaldimedone.