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SESQUITERPENE LACTONES OF *AMBROSIA CORDIFOLIA*

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Key Word Index: *Ambrosia cordifolia*; *Dicoria brandegei*; *Dicoria canescens*; Compositae; sesquiterpene lactones, modified pseudoguaianolides; cordilin; psilostachyin C; psilostachyin; canambrin.

Abstract—Extraction of *Ambrosia cordifolia* (Gray) Payne furnished the modified pseudoguaianolides psilostachyin C (I) and cordilin. The latter was shown to be a stereoisomer IV of psilostachyin (II). *Dicorea brandegei* Gray and *D. canescens* T. &. G. yielded no crystalline lactone components.

INTRODUCTION

IN A review[1] brief reference was made to the isolation of psilostachyin C (I)[2] and a new isomer of psilostachyin (II)[3] from *Ambrosia cordifolia* (Gray) Payne, a 'franserioid' member of the genus.[4] In the following we provide details and discuss the stereochemical differences between the new isomer IV which we have named cordilin, psilostachyin (II)[5] and a third stereoisomer canambrin (III), which has since been isolated from *Ambrosia canescens* (Gray) Payne.[16]

RESULTS

That the new stubstance from *A. cordifolia*, m.p. 210–211°, $[\alpha]_D$ −100°, was stereoisomeric with psilostachyin (II) and canambrin (III)[17] was clear from the empirical formula, the IR spectrum (hydroxyl at 3550 cm^{-1} and two γ-lactone functions at 1765 cm^{-1}, one of

[1] HERZ, W. (1968) *Recent Advances in Phytochemistry* MABRY, T. J , ALSTON, R. E. and RUNECKLES, V. C. eds.), Vol. 1, p. 229, Appleton–Century–Crofts, New York.
[2] KAGAN, H. B., MILLER, H. E., RENOLD, W., LAKSHMIKANTHAM, M. V., TETHER, L. R., HERZ, W. and MABRY, T. J. (1966) *J. Org. Chem.* **31**, 1629.
[3] MABRY, T. J., MILLER, H. E., KAGAN, H. B. and RENOLD, W. (1966) *Tetrahedron* **22**, 1139.
[4] PAYNE, W. W. (1964) *J. Arnold Arb.* **45**, 401.
[5] Psilostachyin has so far been isolated from *A. arborescens* Mill.,[6] *A. pumila* (Nutt) Gray,[6] *A. tenuifolia* Spreng.,[6] and from certain collections of *A. artemisiifolia* L.,[7,8] *A. confertiflora* DC,[6,9] *A. cumanensis* HBK[9–12] *A. psilostachya* DC,[2,3,9,11–14] and *Hymenoclea monogyra* T. & G.[15]
[6] HERZ, W., ANDERSON, G. A., GIBAJA, S. and RAULAIS, D. (1969) *Phytochemistry* **8**, 877.
[7] MABRY, T. J. (1970) in *Phytochemical Phylogeny* (HARBORNE, J. B. ed.), pp. 269–300, Academic Press, London.
[8] BIANCHI, E., CULVENOR, C. C. and LODER, J. W. (1968) *Australian J. Chem.* **21**, 1109.
[9] YOSHIOKA, H., RENOLD, W., FISCHER, N. H., HIGO, A. and MABRY, T. J. (1970) *Phytochemistry* **9**, 823.
[10] ROMO, J., ROMO DE VIVAR, A., DIAZ, E., VELEZ, A., LEON, E. and URBINA, F. (1970) *Recent Advances in Phytochemistry* (STEELINK, C. and RUNECKLES, V. C., eds.), Vol. 3, p. 249, Appleton–Century–Crofts, New York.
[11] MILLER, H. E., MABRY, T. J., TURNER, B. L. and PAYNE, W. W. (1968) *Am. J. Botany* **55**, 316.
[12] MILLER, H E. and MABRY, T. J. (1967) *J. Org. Chem.* **32**, 2929.
[13] MABRY, T. J , RENOLD, W., MILLER, H. E. and KAGAN, H. B. (1966) *J Org. Chem.* **31**, 681.
[14] YOSHIOKA, H. and MABRY, T. J. (1969) *Tetrahedron* **25**, 4767.
[15] TORIBIO, F. P. and GEISSMAN, T. A. (1969) *Phytochemistry* **8**, 313.
[16] ROMO, J. and RODRIGUEZ-HAHN, L. (1970) *Phytochemistry* **9**, 1610.
[17] The physical properties reported for canambrin, m.p. 209–210°, $[\alpha]_D$ −134°, were very similar to those of cordilin. However, direct comparison of cordilin and a sample of canambrin kindly supplied by Dr. A. ROMO DE VIVAR established their non-identity.

which was α,β-unsaturated because of the UV max at 210 nm), from the NMR spectrum which was very similar to that of II and III (see Table 1) and from the chemical transformations (catalytic hydrogenation to a mixture of isocordilin (V) and dihydrocordilin (VI), $NaBH_4$ reduction to VI). Dehydration of V with thionyl chloride-pyridine afforded a compound which possessed the properties of VII; dehydration of VI resulted in a mixture of VII and IX. MS of psilostachyin, canambrin and cordilin were essentially superimposable and displayed only minor differences in the relative intensities of a few peaks.

TABLE 1. NMR SPECTRA OF CORDILIN AND RELATED COMPOUNDS*

Compound	C-5 Me	C-10 Me	H-6	H-7	H-13
II†	1·22	1·04*d* (7·1)	4·96*d* (10)	3 4*m*	5·53*d* (3) 6·29*d* (3)
II‡	1·23	1·05*d* (7·4)	4·98*d* (9)	3·4*m*	5·51*d* (3 2) 6 29*d* (3 7)
III†,§	1·28	1·20*d* (7)	4 73*d* (10)	?	5·58*d* (3) 6 28*d* (3)
IV†	1·25	1·18*d* (7·5)	4·65*d* (9·5)	3 33*m*	5·60*d* (2·5) 6 22*d* (3 0)
IV‡	1·27	1 19*d* (7 1)	4 71*d* (9 9)	3·33*m*	5·57*d* (3 1) 6·29*d* (3 2)
X†,¶	5 12*d* (1·5) 5 65*d* (2)	0·96*d* (7)	5·28*dt* (8,2)	3·33*m*	5·56*d* (2) 6 29*d* (2 5)
XI†‖	1·68	1 00*d* (6·5)	5·44*d* (9)	3 33*m*	5 60*d* (3) 6 29*d* (3)

* Run in $CDCl_3$ solution using TMS as internal standard at 60 MHz on a Varian A-60 and at 90 MHz on a Bruker HFX-10 NMR spectrometer. Values are in ppm, multiplicities are indicated by the usual symbols: *d*—doublet; *t*—triplet, *m*—multiplet whose center is given. Unmarked signals are singlets. Figures in parentheses are line separations. Slight differences in chemical shifts are due to small calibration errors; small differences in line separation are due to better resolution at 90 MHz and a more accurate method of determining frquencies.

† At 60 MHz.
‡ At 90 MHz.
§ Values taken from Ref. 16.
¶ Values taken from Ref. 3.
‖ Acetate at 2 08 ppm.

The relative and absolute stereochemistry of psilostachyin has been established unequivocally by correlation with coronopilin which in turn has been correlated with parthenin and ambrosin of authenticated structure.[18] The path by which canambrin was correlated[16] with psilostachyin indicated that the two substances differed only in the stereochemistry of the saturated γ-lactone ring, i.e. at C-1. Hence we suspected that cordilin differed from psilostachyin at C-5 or at C-1 and C-5 both.

The CD curves of psilostachyin and cordilin (Fig. 1) were very similar and exhibited a negative Cotton effect near 250 nm which can be ascribed to the unsaturated lactone chromophore,[19] whereas the curve of canambrin displayed only an inflection near 250 nm and a minimum at 230 nm. In the case of II and IV, the shape of the curves is satisfactorily explained by assuming superposition of a negative Cotton effect due to the n,π^* transition of the unsaturated lactone function at 250 nm on a positive Cotton effect near 225 nm due to the saturated lactone, while in the case of canambrin, the curve can be rationalized by

[18] EMERSON, M. T., CAUGHLAN, C. N and HERZ, W. (1966) *Tetrahedron Letters* 3151.
[19] STOCKLIN, W , WADDELL, T G. and GEISSMAN, T. A. (1970) *Tetrahedron* **26,** 2397.

assuming that two negative Cotton effects near 250 and 225 nm are superimposed. This indicates that the chirality of the unsaturated lactone function in all three compounds is the same, i.e. that the unsaturated lactone ring of cordilin is *cis*-fused and β-oriented as is that of psilostachyin and canambrin, that the stereochemistry of the saturated lactone ring of canambrin is opposite to that of psilostachyin as already inferred on different grounds,[16] and that the stereochemistry of cordilin (IV) and psilostachyin (II) at C-1 is the same.

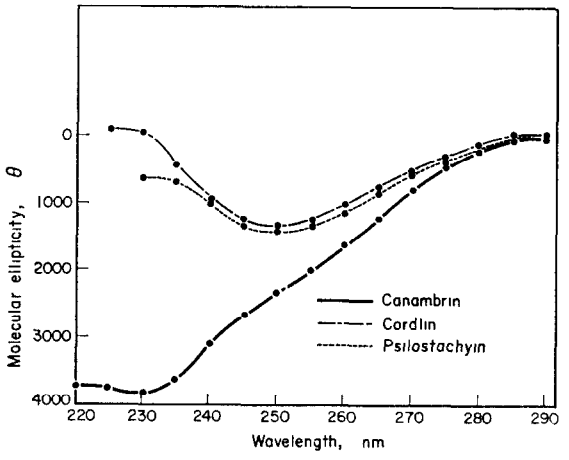


FIG. 1. CD CURVES OF PSILOSTACHYIN, CANAMBRIN AND CORDILIN.

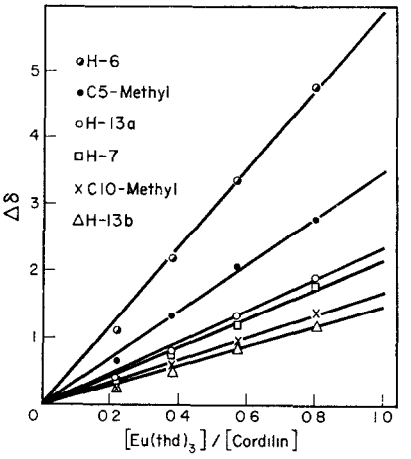


FIG. 2. EFFECT OF EUROPIUM TRIS(2,2,6,6-TETRA-HYDRO METHYLHEPTANE-3,5-DIONATE) ON THE NMR SPECTRUM OF CORDILIN.

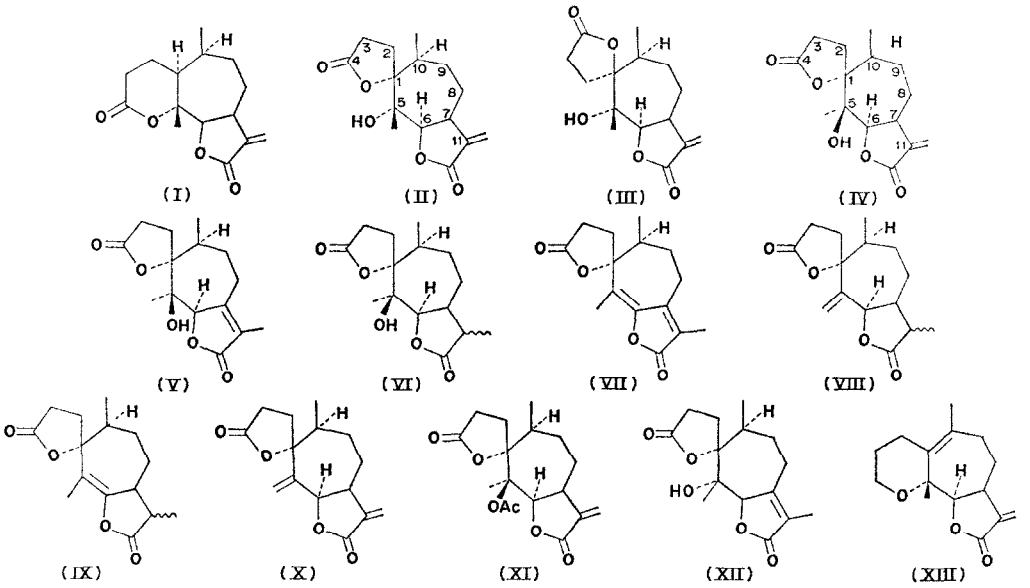
Hence II and IV must differ from each other either at C-5 or at C-10 or at both of these centers. On biogenetic grounds, the C-10 methyl group of cordilin should be β as in all other sesquiterpene lactones of *Ambrosia* species; hence II and IV should differ from each other at C-5. Obviously dehydration of cordilin to the known anhydropsilostachyin (X)[3] would have been a means of verifying this deduction; however, a number of attempts to effect this conversion were entirely unsuccessful (see Experimental). Eventually, treatment of IV with acetic anhydride–toluenesulfonic acid gave the gummy acetate XI in good yield (80%). Attempts to pyrolyze XI at low temperatures (300–375°) resulted in recovery of starting material. Pyrolysis at 450° gave only carbonaceous material, but injection of XI into a vapor phase chromatograph with the injection port at 475° gave a peak (2–3% of total) which had the same retention time as authentic X.

Examination of the NMR spectra of the three stereoisomers (Table 1) lends further support to the proposed structure IV for cordilin. It is known that protons in the vicinity of hydroxyl or ether oxygen but not bonded to the same carbon atom are deshielded to some extent although the mechanism for the deshielding is not clear.[20] In accordance with expectations, the chemical shifts of the C-5 methyls in II, III and IV are very nearly the same since the C-5 methyl group is close to the tertiary hydroxyl in all instances (models) and the effect of the lactone oxygen is negligible in comparison. On the other hand the C-10 methyl groups are in quite different environments, the C-10 methyls of III and IV being

[20] JACKMAN, L. M. and STERNHELL, S. (1969) *Applications of Nuclear Magnetic Resonance in Organic Chemistry*, 2nd Edn, Pergamon Press, Oxford.

considerably closer to an oxygen atom than the C-10 methyl of II. Thus, the signal of the C-10 methyl group of II is expected to be at higher field than that of III or IV, as is indeed found to be the case.

Similarly, since H-6 of psilostachyin is affected by two oxygen atoms, H-6 of canambrin by one nearby oxygen atom and H-6 of cordilin by only one more distant oxygen atom (models), the order of chemical shifts for the H-6 signal should be II > III > IV, in accordance with observations (Table 1). Moreover, examination of a model of XI reveals that in most of the rotameric forms H-6 and the C-5 methyl are in the deshielding cone of the acetate carbonyl, while the C-10 methyl group is in the shielding cone. This leads to the prediction that the signals of H-6 and of the C-5 methyl group should occur at lower field in XI than in IV and that the C-10 methyl signal should be at higher field in XI than in IV. Examination 7 of the data of Table 1 shows that this is so.



Lastly the results obtained by use of the chemical shift reagent $Eu(thd)_3$ are fully consonant with the proposed stereochemistry. Cordilin has only one group, the hydroxyl, which can complex strongly with lanthanide shift reagents; the effect on chemical shifts due to complex formation at the lactone group is expected to be considerably smaller. Measurements on the model indicate that if this assumption be correct the H-6 and the C-5 methyl signals should experience the largest chemical shifts on complexation with $Eu(thd)_3$ and that the H-7, H-13 and C-10 methyl signals should experience only small to moderate chemical shifts. Figure 2 demonstrates that the results are in accordance with the predictions.

The difference in the ease with which II, III and IV undergo dehydration (*vide supra*) is also explained by examination of the models. The tertiary hydroxyl group of IV is flanked on both sides by bulky groups which interfere greatly with reagent approach, while in psilostachyin and canambrin one of the bulky groups is on the opposite side of the ring, thus making the hydroxyl group of II or III much more accessible than that of IV. Likewise rotation of the acetate function of XI to the orientation required for pyrolytic *cis*-elimination is rendered extremely difficult. On the other hand, bimolecular elimination of V to VII

proceeds relatively easily, presumably because the stereoelectronic requirements (hydroxyl and H-6 *trans*) are satisfied and possibly also because the presence of unsaturation at $\Delta^{7(11)}$ alters the steric requirements, whereas under similar conditions, XII, where the hydroxyl group and H-6 are *cis*, could not be converted to VII.

After completion of our work, Higo *et al.*[21] reported the isolation of psilostachyin B(XIII)[22] from *A. cordifolia* collected near Tucson, Arizona. No other lactones were characterized. Our own material, whether from Sonora State, Mexico, or Tucson, Arizona, reproducibly furnished cordilin as the major crystallizable constituent.

In the course of our continuing study of Ambrosiinae, we have also examined extracts of *Dicorea brandegei* Gray and *D. canescens* T. & G., the only representatives of this previously uninvestigated genus. No homogeneous sesquiterpene lactone components could be isolated.

EXPERIMENTAL

M.ps are uncorrected. Rotations were run in $CHCl_3$, UV spectra in ethanol, IR spectra as KBr pellets and CD curves in MeOH on a Jasco ORD/UV-5 recording spectrophotometer. MS were run at 70eV on a MS-902 mass spectrometer. Analyses were performed by F. Pascher, Bonn, Germany.

Isolation of psilostachyin C and cordilin. Above-ground parts of *Ambrosia cordifolia* (Gray) Payne, wt. 2·7 kg, collected by R. J. Barr on 26 February 1965, 1 mile west of Minas Nuevas, Sonora, Mexico (Barr No. 65–113, on deposit in herbarium of Florida State University) was extracted in the usual fashion.[23] The crude gum, wt 16·2 g, was chromatographed over 300 g of silicic acid (Mallinckrodt 100 mesh), 200 ml fractions being collected. Constituents were monitored by TLC. C_6H_6 and C_6H_6–$CHCl_3$ (4:1, 3:2, 2·3 and 1:4) eluted gummy mixtures. $CHCl_3$ and $CHCl_3$–MeOH (99:1) eluted solid material and some gum. The first such fractions contained psilostachyin-C (I), wt 0·8 g, m.p. 225–227° after several recrystallizations from acetone–hexane, which was identified by IR and NMR spectrum, rotation and direct comparison with an authentic sample. The later fractions contained 3·7 g of cordilin (IV), m.p. 210–211° after recrystallization from acetone–hexane, $[\alpha]_D$ −100° (*C.* 0·105), IR spectrum 3550 (hydroxyl), 1765 (double intensity, γ-lactones) and 1660 cm^{-1} (conjug. double bond), NMR signals (DMSO-d_6) at 6·02*d* and 5·58*d* (3, exocyclic methylene), 4·67*d* (9, H-6), 3·2*m* (H-7), 1·08 (C-5 methyl) and 1·01*d* (7, C-10 methyl), UV λ_{max} 210 nm (ϵ 15100). (Calc. for $C_{15}H_{20}O_5$: C, 64·27; H, 7·19; O, 28·54; MW 280. Found: C, 64·43; H, 7·38; O, 28·39, MW-MS 280). A m.m.p. with authentic canambrin was depressed. The more polar fractions gave gums. Extraction of *A. cordifolia* collected on 12 September 1965 (Barr No. 65-277) and on 5 May 1967 (Barr No. 67-160) along the edges of Pima Canyon, Catalina Mountain foothills near Tucson, Arizona, gave very similar results.

Hydrogenation of cordilin. A solution of 0·5 g cordilin in 70 ml EtOH was hydrogenated with 5% Pd–C catalys at atmospheric pressure until H_2 uptake ceased. TLC established the presence of two substances which were separated by chromatography over 50 g of silica gel—10% $AgNO_3$ activated at 125° for 1 hr. $CHCl_3$–MeOH (99:1) eluted first isocordilin (V), m.p. 241°, UV λ_{max} 224 nm (15 200), IR 3600 (hydroxyl) 1760 br (strong, two lactones) and 1665 cm^{-1} (conjug. double bond), NMR signals ($CDCl_3$) at 4·9 br (H-6), 1·8*d* (2, C-11 methyl), 1·11*d* (7, C-10 methyl), and 1·09 (C-5 methyl). The existence of allylic coupling between H-6 and the vinyl methyl group was demonstrated by spin decoupling (Calc. for $C_{15}H_{20}O_5$: C, 64·27; H, 7·19; O, 28·54. Found: C, 64·60; H, 6·92; O, 28·40%). Subsequent fractions eluted dihydrocordilin (VI), m.p. 221–223°, $[\alpha]_D$ −16°, IR 3600 (hydroxyl) and 1775 cm^{-1} (strong, two δ-lactones, NMR signals (DMSO-d_6) at 4·50*d* (7, H-6), 1·31 (C-5 methyl), 1·02*d* and 0·96*d* (7, C-10 and C-11 methyl). (Calc. for $C_{15}H_{22}O_5$: C, 63·81; H, 7·85; O, 28·34. Found: C, 63·99; H, 7·83; O, 27·93%). Dihydrocordilin was also obtained by $NaBH_4$ reduction of cordilin and chromatography of the gummy product.

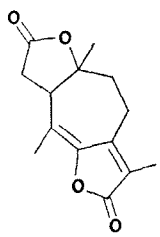
Dehydrations. (a) A mixture of V and VI, obtained by catalytic hydrogenation of 0·5 g of cordilin, was dissolved in 35 ml of pyridine and allowed to stand with 2·5 g of $SOCl_2$ at 0° for 1 hr. After the usual work-up the gummy product was chromatographed over 50 g of silicic acid. C_6H_6–$CHCl_3$ (1:1) eluted solid anhydroisocordilin (VII) which was recrystallized from acetone–hexane, yield 0·1 g, m.p. 167–170°, IR 1750 br (two δ-lactones), 1640 and 1610 cm^{-1} (two double bonds), UV λ_{max} 285 nm, NMR signals ($CDCl_3$) at 1·98 *br* and 1·86 *br* (C-5 and C-11 methyls) and 1·08*d* (C-10 methyl). (Calc. for $C_{15}H_{18}O_4$: C, 68·68; H 6·92; O, 24·40. Found: C, 68·67; H, 7·01; O, 24·48%). Further elution gave a mixture of two anhydro dihydrocordilins, presumably VIII and IX (TLC, NMR spectrum) as a gum. Under identical (bimolecular

[21] Higo, A., Hamam, Z., Timmermann, B. N., Yoshioka, H., Lee, J., Mabry, T. J. and Payne, W. W. (1971) *Phytochemistry* **10**, 2241.

[22] Mabry, T. J., Kagan, H. B. and Miller, H. E. (1966) *Tetrahedron* **22**, 1943.

[23] Herz, W. and Hogenauer, G. (1962) *J. Org. Chem.* **27**, 905.

conditions, a mixture of isopsilostachyin (XIII) and dihydropsilostachyin prepared by catalytic hydrogenation[3] of psilostachyin did not undergo dehydration. Treatment of the mixture with $HOAc-H_2SO_4$ (5:1)[3] on the steam bath for 3 hr gave a mixture (TLC, NMR spectrum) of anhydrodihydropsilostachyin (VIII)[3] and a faster-moving dienic fraction (λ_{max} 285 nm) which could be separated by extensive chromatography. The NMR spectrum of this material, m.p. 173–175° (multiplet at 4·68 ppm–intensity slightly less than one proton, broadened vinyl methyl signal at 1·95 and 1·86 ppm, methyl singlet at 1·26 superimposed on methyl doublet at 1·23 ppm–total intensity of the last two signals equivalent to 3 protons) and the analysis (Calc.–for $C_{15}H_{18}O_4$: C, 68·68; H, 6·92; O, 24·40. Found: C, 68·52; H, 6·96, O, 24·42%) indicated the presence of mainly one anhydroderivative different from VII. A plausible structure for the main constituent was XIV, formed as the result of a rearrangement induced by the stringent reaction conditions, if it is assumed that the C-10 methyl group is shielded and H-1 deshielded by one of the double bonds. (b) Attempts to dehydrate cordilin with $SOCl_2$–pyridine, $POCl_3$–pyridine, alumina–pyridine, $HOAc-H_2SO_4$ and other acid combinations resulted either in recovery of starting material or, depending on the vigor of the conditions, in formation of mixtures containing up to 10 constituents (TLC).



(XIV)

Acetylcordilin (XI) 500 mg cordilin, 25 mg toluenesulfonic acid and 25 ml Ac_2O were stirred at room temp. for 2 hr, then at 60° for 2 hr and finally refluxed for 1 hr, the progress of the reaction being monitored by TLC. The solution was cooled, poured over ice and the mixture thoroughly extracted with $CHCl_3$. Evaporation of the washed and dried extract gave 400 mg of gum which could not be crystallized but was homogeneous on TLC, gave the NMR spectrum listed in Table 1, and was used immediately for the pyrolysis experiments briefly described in the Results.

Extraction of Dicoria brandegei *Gray*. Above-ground parts, wt 1·5 kg, collected by R. J. Barr on 30 August 1966, along Arizona Route 64 near Tonalea, Navajo Co., Arizona (Barr No. 66-96A on deposit in herbarium of Florida State University gave 31·4 g of gum which was chromatographed over 750 g of silicic acid in the usual manner, 1 l. fractions being collected. None of the 110 fractions gave homogeneous material. Chromatography of a second extract, 58·8 g of gum, from 3·9 kg of plant, collected by Dr. G. Caple along Highway 89, 2 miles south of Page, Coconino Co., Arizona, gave similar results.

Extraction of Dicoria canescens *T. & G.* Above-ground parts, wt 1·2 kg, collected by R. J. Barr on 16 October 1965, in the bed of the Gila River, near Dome, Yuma Co., Arizona (Barr No. 65-423 on deposit in herbarium of Florida State University) gave 29·5 g of gum which was chromatographed over 600 g of silicic acid in 1 l. fractions. None of the 100 fractions gave homogeneous material.

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