was evaporated to a colorless sirup (125 mg.). The latter was fractionated on a silicic acid column (2 × 25 cm.) using benzene–methanol (10:3) to give 120 mg. of a product (XV) as a homogeneous sirup, which was dissolved in 3.5 ml. of pyridine, cooled to 5°, and treated with 350 mg. of *p*-nitrobenzoyl chloride. The suspension was kept at room temperature overnight and poured into ice–water, and the solid was washed with water, aqueous bicarbonate, and finally with water to give 150 mg. of crude product. Recrystallization from a mixture of acetone, ether, and pentane gave pure material: m.p. 149–150°, $[\alpha]^{24}D$ 15° (*c* 2, chloroform).

Anal. Calcd. for $C_{28}H_{22}N_6O_{14}$: C, 50.50; H, 3.34; N, 12.65. Found: C, 50.79; H, 3.52; N, 12.01.

Acknowledgment.—We thank Mr. C. E. Childs and his staff for the microanalyses and Dr. J. M. Vandenbelt and his staff for the physical measurements.

Nuclear Magnetic Resonance Spectroscopy of Acetylated Methyl Glycopyranosides of Aminohexoses. Characterization of an Aminohexose from Septacidin

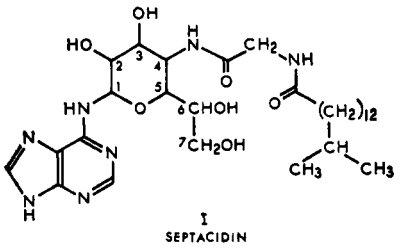
H. AGAHIGIAN, G. D. VICKERS, M. H. VON SALTZA,[1a] JOYCE REID,[1a] A. I. COHEN,[1a] AND H. GAUTHIER[1b]

Olin Mathieson Chemical Corporation, 275 Winchester Avenue, New Haven 4, Connecticut

Received November 9, 1964

Acid hydrolysis of septacidin (1) yielded a monoaminoheptose which was subsequently converted to the monoaminohexose. Chemical proof demonstrated the aminohexose was a 4-amino-4-deoxy-L-glucose. N.m.r. study of the β anomer of the methyl glycoside tetraacetate derivative (2) of this aminohexose, utilizing proton–proton spin decoupling at 60 Mc. and solvent effect studies at 100 Mc., established the position of the acetamido group and the configuration of the ring protons. The 100-Mc. spectra of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-β-L-glucopyranoside (2) in deuteriochloroform and acetonitrile exhibited selective solvent–solute interactions.

The cytotoxic and antifungal agent, septacidin (1),[2] contains a monoaminoheptose moiety, $C_7H_{15}NO_6$.[3] Periodate oxidation of a suitable derivative of this



I
SEPTACIDIN

aminoheptose cleaved off a terminal hydroxymethyl group, and subsequent reduction with sodium borohydride followed by acidic hydrolysis yielded a monoaminohexose, $C_6H_{13}NO_5$. The crystalline β anomer of the methyl glycoside tetraacetate derivative of this aminohexose was subjected to n.m.r. spin-decoupling studies.

Prior to this work, chemical studies and n.m.r. spectra of derivatives of the aminohexose indicated that this sugar had an unbranched aldose structure with the amino group at position 3 or 4 and suggested that it had a *gluco* or *ido* configuration. Thus, these proton decoupling studies sought (1) to establish the position of the amino group in the aminohexose and (2) to determine the configuration of this sugar.

Simultaneously with this examination by n.m.r., a chemical proof of the structure and configuration of this aminohexose was obtained by a comparison of the physical properties of the α anomer of the methyl glycoside tetraacetate derivative with those of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-α-D-glucopyranoside.[4a] These two compounds were identical (melting point, $[\alpha]D$, infrared and n.m.r. spectra, and X-ray diffraction pattern) except for the sign of rotation.[4b] Thus, the aminohexose was shown to be 4-amino-4-deoxy-L-glucose. This conclusion agrees with that arrived at by the n.m.r. studies described below.

Since previous n.m.r. studies[5-16] have demonstrated the utility of this spectroscopic technique in carbohydrate chemistry, the configuration of the ring protons and the positions of the various substituents of the methyl glycoside tetraacetate derivative 2 of the aminohexose were established by these methods, *i.e.*, proton–proton spin decoupling and the use of different solvents at 60 Mc. and 100 Mc. The magnitude of the coupling constant[5,6,17] was the criterion for determining whether adjacent methine protons on carbons 1 through 5 are coupled axial–axial or axial–equatorial. Although the possibility of the methyl glycoside tetraacetate derivative 2 being a furanose structure does exist, the proton n.m.r. spectrum indicates the pyranose

(1) (a) The Squibb Institute for Medical Research, New Brunswick, New Jersey. (b) Varian Associates, Palo Alto, Calif.

(2) J. D. Dutcher, M. H. von Saltza, and F. E. Pansy, *Antimicrobial Agents Chemotherapy*, **83** (1963).

(3) M. H. von Saltza, J. D. Dutcher, and J. Reid, Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964, p. 15Q. The asymmetric center at C-6 in the aminoheptose moiety of septacidin has the L-configuration and is 4-amino-4-deoxy-L-glycero-L-glucoheptose.

(4) (a) This sample was kindly provided by Dr. E. J. Reist, Stanford Research Institute, Menlo Park, Calif. The unequivocal synthesis of this compound has been reported: E. J. Reist, R. R. Spencer, B. R. Baker, and L. Goodman, *Chem. Ind.* (London), 1794 (1962). (b) M. H. von Saltza, J. Reid, and J. D. Dutcher, in press.

(5) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **79**, 1005 (1957); **80**, 6098 (1958).

(6) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); M. Karplus and D. H. Anderson, *ibid.*, **30**, 6 (1959).

(7) P. K. W. Woo, H. W. Dion, and L. F. Johnson, *ibid.*, **84**, 1066 (1962).

(8) L. D. Hall and L. Hough, *Proc. Chem. Soc.*, 382 (1962).

(9) O. Ceder, G. Eriksson, J. M. Waisvisz, and M. B. Van der Hoeven, *Acta Chem. Scand.*, **18**, 98 (1964).

(10) A. C. Richardson and K. A. McLauchlan, *J. Chem. Soc.*, 2499 (1962).

(11) R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLauchlan, *ibid.*, 3699 (1962).

(12) V. W. Hofheinz and H. Grisebach, *Z. Naturforsch.*, **17b**, 355 (1962).

(13) D. Horton, *J. Org. Chem.*, **29**, 1776 (1964).

(14) B. Capon and B. E. Connett, *Tetrahedron Letters*, **No. 22**, 1391 (1964).

(15) L. D. Hall and L. F. Johnson, *Tetrahedron*, **20**, 883 (1964).

(16) G. E. McCasland, S. Furuta, L. F. Johnson, and J. N. Shoolery, *J. Org. Chem.*, **29**, 2354 (1964).

(17) H. Conroy, *Advan. Org. Chem.*, **2**, 265 (1962).

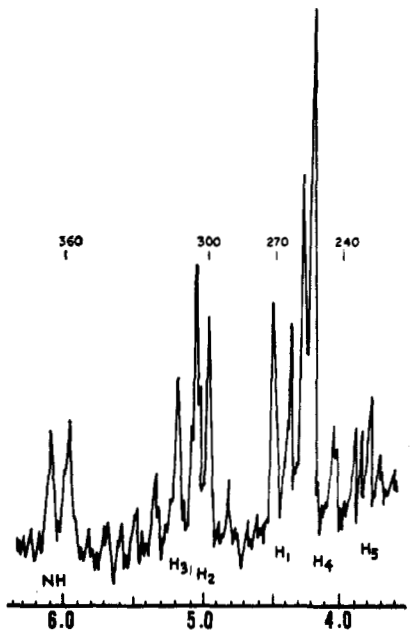


Figure 1.—N.m.r. spectrum (60-Mc.) of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-β-L-glucopyranoside in chloroform-*d*.

ring is present.[18] The n.m.r. spectrum of β-D-glucopyranose pentaacetate[5] exhibited similarities to that of the pentaacetyl aminohexose which was isolated from the septacidin degradation.

In addition to the NH doublet (6.10 p.p.m.), the proton spectrum (Figure 1) of the methyl glycoside tetraacetate derivative **2** contained another doublet (4.45 p.p.m.) which was not further coupled.[19] The chemical shift of the latter doublet substantiated the aldose structure.[5] By comparing the n.m.r. spectrum of the aminohexose derivative **2** with the spectrum of the pentaacetyl of α-D-glucosamine,[13] still further evidence was obtained for the validity of the assignment of the H-1 doublet. Since the other methine protons of the aminohexose derivative **2** have complex fine structure, the H-1 doublet can be conclusively assigned. The assignment of the various methine protons was accomplished by comparing the spectra of model compounds. By means of deuterium exchange, the NH proton and the methine proton on the adjacent carbon atom were assigned. Since the low-field protons (5.08 p.p.m.) had an integral ratio equivalent to two protons, the reasonable assumption was made that these methine protons were on carbon atoms containing acetoxy groups.

The doublet centered at 4.45 p.p.m. was collapsed into a single line by irradiating 30 c.p.s. to lower field (the high-field side of the multiplet centered at *ca.* 5.08 p.p.m.). This multiplet was assigned to the methine protons on the carbons bearing the acetoxy groups.[5,20] Since the coupling constant was 8.5 c.p.s., the methine protons at positions C-1 and C-2 are diaxial, and, since the irradiating frequency was 30 c.p.s. to lower field (*ca.* 5.00 p.p.m.), the C-2 position must contain an acetoxy group. The conclusion was also reached that the position of the acetamido group was limited to either position C-3 or C-4.

In order to establish the position of the acetamido group, the multiplet at 5.08 p.p.m. was observed while irradiating at high field. When the irradiating field had a value of 75–50 c.p.s. (*ca.* 4.25 p.p.m.), considerable deformation of the low-field portion of the 5.08 p.p.m. multiplet was seen. The high-field portion of the multiplet (5.08 p.p.m.) was perturbed at an irradiating field of 30 c.p.s. By decoupling the >NH proton from the ring proton, the significance of these values was demonstrated. When the irradiating field had a value of 105 c.p.s. higher field, the >NH doublet collapsed into a single line. The resonance position of the methine proton on the carbon containing the acetamido group was established as being *ca.* 4.25 p.p.m. Since this resonance position (4.25 p.p.m.) was in the same region as the proton coupled to the low-field proton of the acetoxy multiplet (5.08 p.p.m.), it was concluded that the protons were identical. The deformation of only the low-field portion of the methine multiplet at 5.08 p.p.m., while irradiating at 4.25 p.p.m., suggests the amido function cannot be at C-3, but must be at C-4 and adjacent to just one of the acetoxy groups. If the amido function were at C-3, irradiating at 4.25 p.p.m. would cause collapse of both the low-field and high-field portions of the 5.08-p.p.m. multiplet.

In further decoupling experiments, the collapse of two lines at 4.22 and 4.28 p.p.m. into a singlet when the irradiating field had a high-field value of 36 c.p.s. demonstrated the two methylene protons were coupled to a methine, H-5, at approximately 3.75 p.p.m. Analysis of the spectrum indicated the C-5 proton was coupled to the two methylene protons (4 c.p.s.) and further coupled to the C-4 proton, the value being 10.3 c.p.s. Thus, within the limits of the decoupling experiment,[15] the chemical shifts of each of methine protons and the coupling constants were obtained. The magnitude of the coupling constants indicated that the methine protons H-1 through H-5 are axial.

Further confirmation of the structure was obtained by studying the proton spectrum of compound **2**, which contained the deuterium-substituted NH, at 100 Mc. in different solvents (Figure 2). The multiplet at 5.08 p.p.m. (Figure 1), which was assigned to the methines of the acetoxy-bearing carbon atoms, exhibited a dramatic change when observed in acetonitrile at 100 Mc. The H-5 proton was better resolved when observed in deuteriochloroform at 100 Mc. and clearly exhibited the presence of a doublet further split into triplets (the methylene protons are not necessarily equivalent). In addition, the resonance of the C-4 methine of the deuterium-exchanged product was better resolved in deuteriochloroform and indicated large coupling, 8–10 c.p.s.

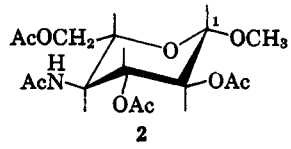
Therefore, the aminohexose studied has a *gluco* configuration and, since the amino group is at the C-4 position, the amino sugar is identified as 4-amino-4-deoxyglucose. The specific rotation showed that it was an L sugar, and the actual derivative used in this study was methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-β-L-glucopyranoside (**2**).

(18) R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961).

(19) The NH doublet was identified by means of a base-catalyzed deuterium exchange and also by noting the effect of concentration on the chemical shift.

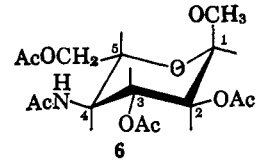
(20) The chemical shift of the methine protons, which were on the carbons bearing the acetoxy groups, was established by comparing them with the resonance positions in the spectrum of the pentaacetyl derivative of 2-amino-2-deoxy-α-D-glucopyranose, as well as other derivatives reported by Lemieux, *et al.*[5]

CHART I



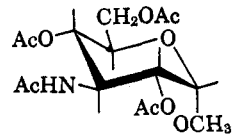
2

methyl 4-acetamide-4-deoxy-2,3,6-tri-O-acetyl-β-L-glucopyranoside



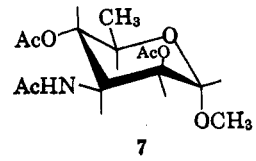
6

methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-α-L-glucopyranoside



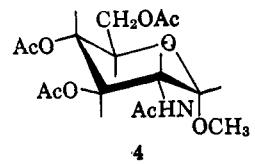
3

methyl 3-acetamido-3-deoxy-2,4,6-tri-O-acetyl-α-D-glucopyranoside



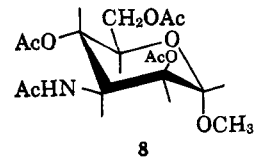
7

methyl 3-acetamido-3,6-dideoxy-2,4-di-O-acetyl-α-D-mannopyranoside



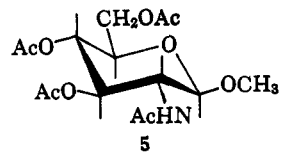
4

methyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranoside



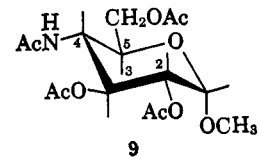
8

methyl 3-acetamido-3-deoxy-2,4,6-tri-O-acetyl-α-D-mannopyranoside



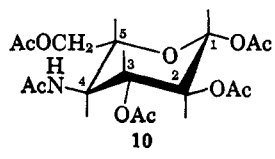
5

methyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside



9

methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-α-D-glucopyranoside



10

4-acetamido-4-deoxy-1,2,3,6-tetra-O-acetyl-β-L-glucopyranose

A correlation study (Table I) of a series of model compounds (Chart I) indicated the consistent nature of the chemical shifts of methyl glucopyranosides and related compounds. Of the acetamido derivatives studied, the resonances of the axial and equatorial methoxyls differed by approximately 6 c.p.s., with the axial being at slightly higher field,[21] although the values of the resonance positions agreed with other reported data.[22] The H-1 proton had a high-field resonance when its configuration was axial and a low-field resonance when equatorial.[22] The lowest field resonance of H-1 proton in this series is *ca.* 5.00 p.p.m. As expected, the C-1 proton of pentaacetyl-α-D-glucosamine would be at lower field position (Table I). The deshielding effect of neighboring acetoxy groups is seen in the resonance of the H-4 proton. The C-3 proton in methyl 3-acetamido-3-deoxy-2,4,6-tri-O-acetyl-α-D-glucopyrancoside (**3**) has a lower field resonance than the C-4 proton of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-α-L-glucopyranose (**6**). In the former case, the acetamido group is adjacent to two acetoxy groups and, in the latter case, adjacent to only one acetoxy group.

(21) S. A. Barker, J. Homer, M. C. Keith, and L. F. Thomas, *J. Chem. Soc.*, 1538 (1963).

(22) G. J. F. Chittenden and R. D. Guthrie, *ibid.*, 1045 (1964).

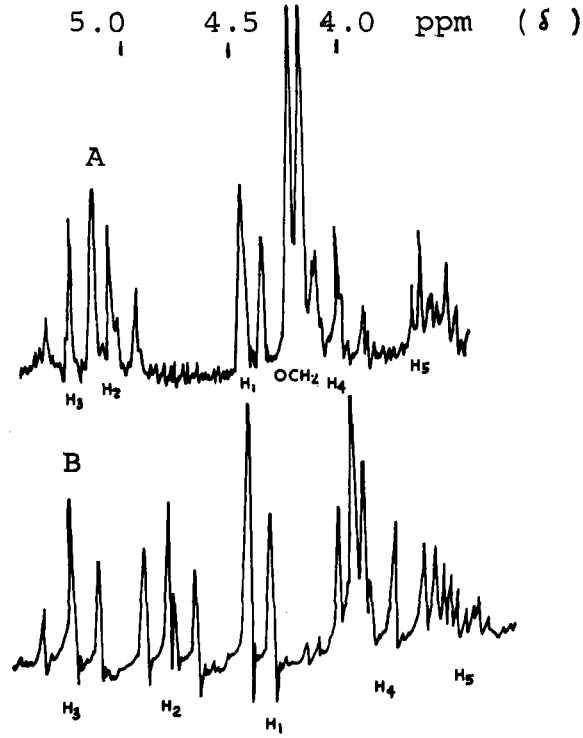


Figure 2.—N.m.r. spectrum (100-Mc.) of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-β-L-glucopyranoside (*NH* deuterium exchanged): (A) in chloroform-*d*; (B) in acetonitrile.

It was observed that the presence of an axial methoxy (or acetyl) group at position 1 causes a downfield shift of about 10 c.p.s. for an axial proton at position 5 (or 3), due to a 1,3-diaxial deshielding effect. The methylene protons appeared at 4.20–4.28 p.p.m.

The assignment of the acetyls has been discussed,[13] and the data exhibited the relationship noted between the chemical shift of the methyl and the configuration.[5] However, in order to establish the assignment of the acetamido methyl, the spectrum of methyl 3-acetamido-3,6-dideoxy-2,4-di-O-acetyl-α-D-manno pyranoside (**7**) was obtained (Table I). The 4-acetyl methyl was at 2.05, the 2-acetyl methyl was at 2.15, while the methyl of the 3-acetamido group was at 1.93 p.p.m. The absence of the C-6 acetoxy methyl confirms the assignment of the acetamido methyl. The methyl resonances of the penta-O-acetyl-β-D-glucopyranose[5] were located at 2.05 and 2.12, but there was no resonance line at 1.93 p.p.m. These data do not appear to be in agreement with recent literature data,[13] but are consistent with the assignment made in a study of 3-amino-1,2-cyclohexanediol derivatives.[23] The spectrum of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-α-D-glucopyranoside (**9**) was compared with that of the β-L stereoisomer (**2**) derived from septacidin. The expected deviations in chemical shifts—especially for the methoxy, H-5, and H-1 protons (coupling constants also changed)—were observed.

(23) F. A. L. Anet, R. A. B. Bannard, and L. D. Hall, *Can. J. Chem.*, **41**, 2331 (1963).

TABLE I

CHEMICAL SHIFTS AND COUPLING CONSTANTS OF CERTAIN AMINOHEXOSE DERIVATIVES[a]

Compd.[b]	H-1	H-2	H-3	H-4	H-5	H-6	NH	OAc	NAc[c]	OMe
2	4.45[d] axial	4.95	5.22	4.20	3.78	4.28	6.10 (d, 8.5)[e]	2.05 2.10	1.95	3.53 equat.
3	4.17 equat.	4.9			→4.00	4.22	5.60 (d, 7.5)	2.05 2.08	1.90	3.45 axial
4	4.75 (d, 3.5) equat.	4.30	5.25	5.08	3.93	4.20	5.82 (d, 9.5)	2.02 2.08	1.95	3.40 axial
5	4.65 (d, 8.0) axial	3.92	5.33	5.08	3.75	4.25	6.12 (d, 8.5)	2.05 2.10	1.97	3.53 equat.
6[f]	5.02 equat.	5.20	5.37	~4.27	3.96	4.27	6.13 (d, 8.5)	2.07 2.08 2.12	1.97	3.43 axial
7[g]	4.65 equat.	4.95	4.67 broad	4.83	3.96		5.80 (d, 8.0)	2.05 2.15, axial	1.93	3.38 axial
8	4.77 equat.	5.08	4.42	4.92	3.93	4.22	5.80 (d, 9.0)	2.18, axial 2.08 2.05	1.93 1	3.42 axial
9	~5.00 equat.	~5.00	5.30	~4.18	3.90	4.22	5.80 (d, 8)	2.10 2.08 2.05	1.95	3.62 axial
10	5.73 axial	~5.23	~5.23	~4.25	3.77	4.23	6.25 (d, 9.0)	2.10 2.08	1.95	

[a] Measured in p.p.m. from internal TMS in $CDCl_3$ solution at 60 Mc. [b] Numbered as in Chart I. [c] Methyl resonances. [d] Midpoint of doublet. [e] Coupling constant for doublet (d). [f] Spectrum is identical with the α-D anomer: Dr. E. J. Reist. [g] The C-5 methyl is a doublet at 1.18 p.p.m.

Experimental

The H^1 n.m.r. spectra were obtained by means of the Varian HR-60 spectrometer at 60 Mc. (14.092 kgauss) and the Varian A-60 spectrometer. The decoupling experiments were done with the NMR Specialties PD-60 homonuclear decoupler. The 100-Mc. spectra were obtained by means of the Varian Associates HA-100 spectrometer. The chemical shifts are measured in cycles per second from internal tetramethylsilane. Decoupled spectra were obtained at first by irradiating at higher field than the observed peak, between −25 and −115 c.p.s. in 3-c.p.s. increments, and then by irradiating the low-field peak while observing the high-field peak. Where decoupling was noted, the system was optimized, and more accurate data were obtained.

Since the amide proton exchanges only slowly in water, the exchange was accelerated by the addition of a base. The spectrum was obtained of 0.4 ml. of a deuteriochloroform solution of the sample. Then 50 μl. of deuterium oxide and 5 μl. of triethylamine were added to the tube and the mixture was shaken several times. The exchange was quantitative, as evidenced by the absence of the amide proton signal.

Osage Orange Pigments. XVI. The Structure of Alvaxanthone[1a]

M. L. WOLFROM, F. KOMITSKY, JR.,[1b] AND P. M. MUNDELL

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received November 17, 1964

Alvaxanthone, a pigment obtained from the root bark of the osage orange (*Maclura pomifera* Raf.), is shown to be 2-(1,1-dimethylallyl)-1,3,5,6-tetrahydroxy-8-(3-methyl-2-butenyl)xanthen-9-one (1).

In a previous paper[2] in this series, we have described the isolation and partial characterization of three new yellow pigments from the root bark of the osage orange (*Maclura pomifera* Raf.). These were tentatively assigned substituted polyhydroxyxanthone structures on the basis of their ultraviolet spectra and various diagnostic tests and were accordingly designated macluraxanthone, osajaxanthone, and alvaxanthone. Subsequently, macluraxanthone and osajaxanthone were shown to be 12-(1,1-dimethyallyl)-5,9,10-trihydroxy-2,2-dimethyl-2H,6H-pyrano[3,2-*b*]xanthen-6-one[3] and 5,8-dihydroxy-2,2-dimethyl-2H,6H-pyrano[3,2-*b*]xanthen-6-one,[1a] respectively.

We wish to report herein the elucidation of the structure of the third osage orange root bark pigment, alvaxanthone.

As reported previously,[2] alvaxanthone, $C_{23}H_{24}O_6$, can be obtained in 0.15% average yield from the dry root bark of the osage orange. Further, acetylation and methylation under mild or severe conditions yielded a bright yellow triacetate and trimethyl ether, respectively. The presence of a fourth hydroxyl, severely hindered and also possibly hydrogen bonded, was indicated by a faint, but definite, alcoholic ferric chloride test on the triacetate.

Although this fourth hydroxyl could not be methylated under the most severe conditions and could not be acetylated under conditions which normally would

(1) (a) Preceding paper in this series: M. L. Wolfrom, F. Komitsky, Jr., and J. H. Looker, *J. Org. Chem.*, **30**, 144 (1965). (b) National Science Foundation Cooperative Graduate Fellow, 1961–1964.

(2) M. L. Wolfrom, E. E. Dickey, P. McWain, A. Thompson, J. H. Looker, O. M. Windrath, and F. Komitsky, Jr., *ibid.*, **29**, 689 (1964).

(3) M. L. Wolfrom, F. Komitsky, Jr., G. Fraenkel, J. H. Looker, E. E. Dickey, P. McWain, A. Thompson, P. M. Mundell, and O. M. Windrath, *ibid.*, **29**, 692 (1964).