Citrus Bitter Principles. IX.[1] Extractives of *Casimiroa edulis* Llave *et* Lex. The Structure of Zapoterin

DAVID L. DREYER[2]

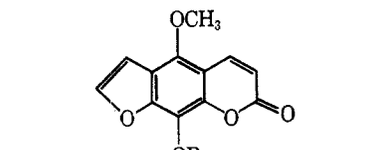
Fruit and Vegetable Chemistry Laboratory,[3] Pasadena, California 91106

Received February 5, 1968

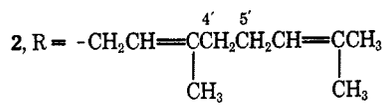
Seed extracts of *Casimiroa edulis* Llave *et* Lex have yielded 5-methoxy-8-geranyloxypsoralen, phellopterin, zapotin, 2′,5,6-trimethoxyflavone, 3′,5,6-trimethoxyflavone, 3′,5,5′,6-tetramethoxyflavone, casimiroin, eduline, edulein, 1-methyl-2-phenyl-4-quinolone, zapoterin, 7α-obacunol, and deacetylnomilin. Zapoterin is a C_{26} limonoid and has been converted into a monoacetate and oxidized to a ketone, zapoterone, with chromic acid. These chemical transformations and spectroscopic considerations indicate that zapoterin is 12α-hydroxyobacunone. The synthesis of 3′,5,6-trimethoxy- and 3′,5,5′,6-tetramethoxyflavone is also reported.

The extractives of *Casimiroa edulis* Llave *et* Lex (Rutaceae) have been the subject of an extensive investigation by Sondheimer and coworkers.[4,5] Our original interest in the limonoids[6] occurring in the seeds of *C. edulis* lead incidentally to a structure proposal for zapotin.[7] Extension of this work has resulted in the isolation of some additional extractives from this source which are reported here. Seed extracts from three different seasons have been studied and the extractives obtained have varied somewhat from year to year.

Chromatography of acetone extracts from *C. edulis* seeds on alumina gave substantial amounts of phellopterin (**1**) and small amounts of a geranyl furocoumarin in the nonpolar eluents. The geranyl coumarin appeared to be homogenous, but it failed to give satisfactory analytical data. It showed an ultraviolet spectrum identical with that of phellopterin (**1**), and mild acid hydrolysis gave 5-methoxy-8-hydroxypsoralen (**3**). The nmr spectrum was very similar to **1** except that upon integration, the ratio of the 4′- and 5′-allyl resonances relative to the methoxy resonance was much too small.



1, R = $-CH_2CH{=}C(CH_3)-CH_3$



2, R = $-CH_2CH{=}C^{4'}H_2C^{5'}H_2CH{=}C(CH_3)-CH_3$

3, R = H

These data suggest that the product isolated is a mixture of phellopterin (**1**) and **2**. The isolation of a similar crystalline complex of imperatorin and 8-geranyloxypsoralen has been reported by Sharma, *et al.*[8] 5-Methoxy-8-geranyloxypsoralen (**2**) was synthesized from geranyl bromide and **3**. It was noncrystalline and slowly decomposed during chromatography on alumina. Superimposition of the nmr spectra of **1** and **2** gave a pattern identical with that of the material isolated from *Casimiroa* extracts.

Coumarins **1** and **2** were not found when the crude plant extracts were worked up with acid according to the original methods of Kincl, *et al.*[4] Repetition of their method gave only 5-methoxy-8-hydroxypsoralen (**3**). On the other hand, no **3** was obtained when the plant extracts were worked up by chromatography. Thus, the occurrence of **3** in *Casimiroa* must be regarded as an artifact. Similar considerations apply to the report of **3** in *Phebalium nudum* (Rutaceae).[9-11]

Fractions eluted from the column with 60–70% benzene in hexane contained mostly zapotin (**5**). Fractions which crystallized after the bulk of the zapotin (**5**) had been removed were a mixture of zapotin and 2′,5,6-trimethoxyflavone (**4**), and substantial amounts of **4** were recovered from the zapotin mother liquors. Flavone **4** was previously reported occurring in the root bark of *C. edulis*.[12] The 2′,5,6-trimethoxyflavone (**4**) present in zapotin appears to be the source of salicylic acid obtained from the base fusion of zapotin reported by Sondheimer and coworkers.[4] Further work-up of the zapotin mother liquors by repeated chromatography gave a new flavone which was eluted slightly behind zapotin and **4**. The new material appeared homogeneous by tlc and exhibited many features resembling those of zapotin. It gave a positive magnesium–HCl test for flavones and turned bright yellow when fumed with hydrogen chloride gas or concentrated hydrochloric acid indicating the presence of a 5-methoxy group. Unlike zapotin and 2′,5,6-trimethoxyflavone, which both show bright light yellow fluorescence under ultraviolet light, the new product showed a dirty yellow fluorescence. The ultraviolet spectrum of the flavone was unlike that of zapotin. It showed the 270-mμ band of 2′,5,6-trimethoxyflavone (**4**) but the long wavelength band occurred at 304 mμ. The nmr spectrum showed complex adsorption in the aromatic region and three methoxy resonances. One of the

(1) Part VIII: D. L. Dreyer, *Tetrahedron*, **24**, 3273 (1968).

(2) Department of Chemistry, San Francisco State College, San Francisco, Calif.

(3) A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(4) F. A. Kincl, J. Romo, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 4163 (1956).

(5) J. Iriarte, F. A. Kincl, G. Rosenkranz, and F. Sondheimer, *ibid.*, 4170 (1956).

(6) D. L. Dryer, *Phytochemistry*, **5**, 367 (1966).

(7) D. L. Dreyer and D. J. Bertelli, *Tetrahedron*, **23**, 4607 (1967). See also P. J. Garratt, F. Scheinmann, and F. Sondheimer, *ibid.*, **23**, 2413 (1967).

(8) Y. N. Sharma, A. Zaman, A. R. Kidwai, R. B. Bates, and V. P. Thalacker, *ibid.*, **22**, 3221 (1966).

(9) L. H. Briggs and R. C. Cambie, *ibid.*, **2**, 256 (1958).

(10) Small amounts of an incompletely characterized severine derivative,[11] which is possibly 4′-dehydrogeranyl-N-benzoyltyramine, were also isolated from the nonpolar eluents of the column. This suggests that the N-benzoyltyramine found in *Casimiroa* by Kincl, *et al.*,[4] is also an artifact formed under the acid conditions employed during work-up. N-Benzoyltyramine was not found in this study.

(11) D. L. Dreyer, *Tetrahedron*, **23**, 4613 (1967).

(12) F. Sondheimer and A. Meisels, *ibid.*, **9**, 139 (1960).

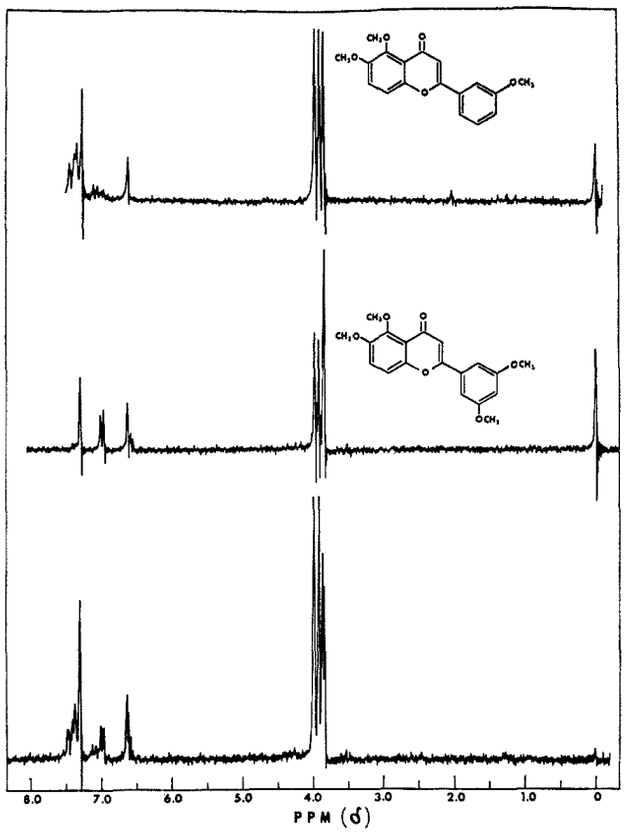
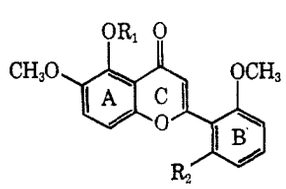
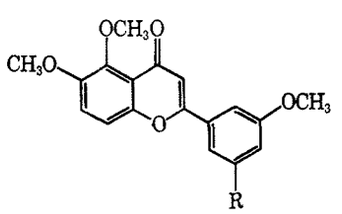


Figure 1.—Top curve, nmr spectrum of synthetic 3′,5,6-trimethoxyflavone (**8**); middle curve, nmr spectrum of synthetic 3′,5,5′,6-tetramethoxyflavone (**9**); bottom curve, nmr spectrum of a natural mixture of **8** and **9** isolated from *C. edulis*.

methoxy resonances did not move upfield when the nmr spectrum was taken in benzene, indicating that it was flanked by two other groups.[13] Mild hydrolysis with hot 20% hydrochloric acid resulted in demethylation of the methoxy group adjacent to the carbonyl group. Demethylation of zapotin (**5**) to the 5-demethyl derivative (zapotinin) (**6**) is conveniently effected under the same conditions.[12] All of the methoxy resonances in the nmr spectrum of the 5-demethylflavone and its derived acetate occurred upfield in benzene relative to chloroform showing that methoxy groups were present in the 5 and 6 positions and that a 3-methoxy group was not present.[13] Resonances for the 7 and 8 protons could be distinguished in the aromatic region of the nmr spectrum. In several solvents these resonances showed the same pattern of chemical shifts in the parent compound, the 5-demethyl derivative and its acetate as was observed for zapotin (**5**), zapotinin (**6**), and zapotinin acetate (**7**). The A ring in the new compound is thus the same as that in zapotin (**5**).



4, R_1 = Me; R_2 = H
5, R_1 = Me; R_2 = MeO
6, R_1 = H; R_2 = MeO
7, R_1 = AcO; R_2 = MeO



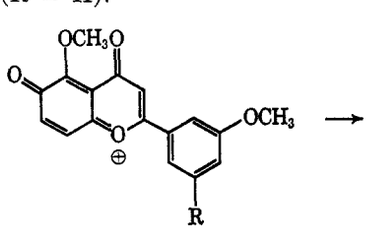
8, R = H
9, R = MeO

A sharp one-proton singlet was apparent in the nmr spectra of the unknown compound (at δ 6.60) and its derivatives. The position of this singlet was well downfield from its extreme upfield position in zapotin[7] and corresponded closely to that of H-3 in a normal flavone. The resonance for the 3 proton in 2′-methoxyflavones (as differentiated from the 2′,6′-dimethoxyzapotins) occurs at about δ 7.00. Thus, the new flavone does not contain a 2′-methoxy group, and the A and C rings are identical with those in zapotin (**5**) and **4**.

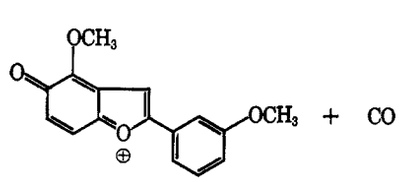
These results were marred by the failure to obtain satisfactory analytical data on the flavone and its derivatives and by several inconsistent bands in the nmr spectrum. The difficulties were resolved by the mass spectrum of the flavone which showed it to be a mixture of a tri- and tetramethoxyflavone. Both base hydrolysis and basic hydrogen peroxide gave 3,5-dimethoxybenzoic acid, indicating the tetramethoxy derivative is 3′,5,5′,6-tetramethoxyflavone. The trimethoxyflavone must be the 3′,5,6-trimethoxy derivative since it is nonidentical with **4**, and 4′,5,6-trimethoxyflavone would have a simple and distinctive nmr spectrum.

Clarification of the situation was achieved by synthesis of **8** and **9**. The nmr spectrum of the natural mixture proved to be a perfect composite of that of the two synthetic flavones (Figure 1). A mixture of the synthetic flavones was unresolved in several tlc solvent systems. The flavone mixture was separated by vpc, the retention times corresponding to those of the synthetic samples.

The mass spectrum of the flavone mixture showed, as in the case of zapotin (**5**),[7] intense M − 15 peaks of both molecular ions which are best represented as structure **10** (R = H and Me). A strong metastable peak at *m/e* 244 corresponds to the conversion, **10** → **11** + CO (R = H).



10



11

The five related flavones found in *C. edulis* are of unusual structure and depart from the usually accepted structural variations. Those of *Citrus* may be considered as typical[14] and generally show structural features suggesting an A ring arising from acetate and a B ring from shikimate. It is not clear if these flavones in *C. edulis* are biogenetically the result of a remarkable

(13) R. M. Horowitz and D. L. Dreyer, unpublished results; see also, R. G. Wilson, J. H. Bowie, and D. H. Williams, *Tetrahedron*, **24**, 1407 (1968); H. M. Fales and K. S. Warren, *J. Org. Chem.*, **32**, 501 (1967).

(14) J. B. Harborne, "Comparative Biochemistry of the Flavonoids," Academic Press, New York, N. Y., 1967, p 175.

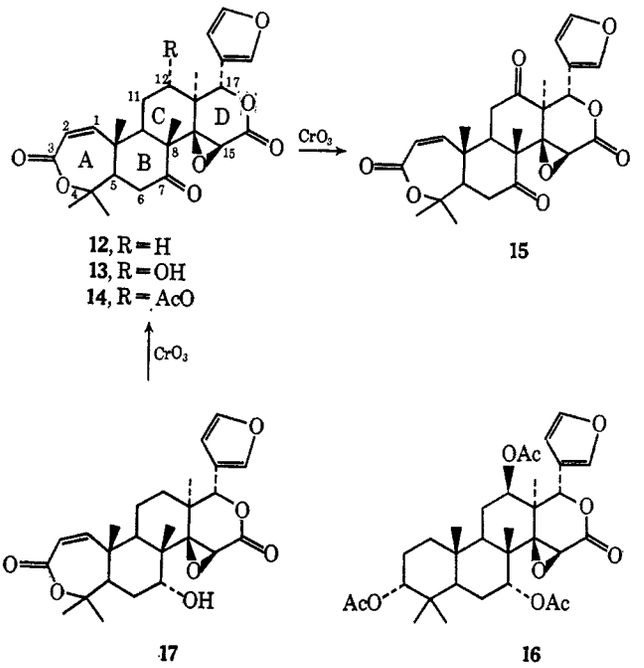
oxidation–reduction sequence or if they are derived by some departure from the usual acetate–shikimate pathway.[15]

Fractions eluted from the column with increasing amounts of benzene in chloroform gave successively casimiroin,[16] edulein (1-methyl-2-phenyl-7-methoxy-4-quinolone),[17,18] eduline (1-methyl-2-phenyl-6-methoxy-4-quinolone),[19] and small amounts of 1-methyl-2-phenyl-4-quinolone.[20] The last is a metabolite previously found in *Lunasia amara* Blanco (Rutaceae),[20] while edulein was previously reported from the bark of *C. edulis*[5] and from *Lunasia quercifolia* (Warb.) Lauterb. *et* K. Schum.[18]

The isolation of zapoterin was reported in the original work of Kincl, *et al.*,[4] on *C. edulis*. In this study zapoterin has been encountered only in extracts of seeds from one of the three seasons studied. Zapoterin tasted bitter and was obviously a C_{26} limonoid.[21] It gave a positive Ehrlich's test indicating the presence of a furan ring.[22,23] Its infrared spectrum showed a hydroxy band and three well-resolved carbonyl bands, as well as bands due to a β-substituted furan ring.[24] The nmr spectrum showed resonances assignable to a β-substituted furan ring, a H-17 furfurylic singlet, an H-15 epoxy proton, an AB doublet identical with that of H-1 and H-2 of obacunone (**12**), and five C-methyl groups.[25] In addition, the nmr spectrum showed a one-proton triplet at δ 4.97. The ORD curve of zapoterin was similar to that of obacunone (**12**)[1] providing substantial evidence for the presence of a 7-keto group. The sum of this spectroscopic evidence indicates that zapoterin is a hydroxyobacunone derivative.

Acetylation of zapoterin with refluxing acetic anhydride–pyridine gave a monoacetate.[26] The nmr spectrum of zapoterin acetate was very similar to that of zapoterin except that the one-proton triplet moved downfield, indicating that zapoterin is a secondary alcohol. Assuming an obacunone (**12**) ring system the hydroxy group must be located at C-11 or C-12. A hydroxy group at C-6 would cause H-6 to be either a singlet or doublet depending on its stereochemistry.[25] Moreover, a 6-hydroxy might cause the ORD curve of zapoterin, as an α-hydroxy ketone, to be somewhat different from that of obacunone (**12**). As the carbinol resonance is a triplet, it is best accommodated at the 12 position with the two adjacent protons at the 11 position.

Chromic acid oxidation of zapoterin gave a diketone, zapoterone (**15**), providing chemical evidence for the presence of a secondary alcohol group in zapoterin (**13**). The ultraviolet spectrum of zapoterone (**15**) indicated that it was neither an α diketone nor a diosphenol, showing that the hydroxy group was not located at C-6.[27]



The stereochemistry at C-12 remains to be considered. A similar problem has been encountered in defining the stereochemistry at C-12 in nyasin (**16**).[28] In the nyasin case it was argued that a 12β-acetoxy group would cause a downfield shift to the 8- and 10-C-methyl resonances in the nmr. The H-12 resonance in either configuration could be a triplet by distortion of the C ring. A 12α-hydroxy group in **13** would account for the relative downfield position of the acetoxy group at δ 2.18 in **14** due to its 1,3 relationship to the furan ring so that it falls in the deshielding region of the furan ring. Accepting the argument that a 12β-acetoxy group should cause a downfield shift to the 8- and 10-methyl resonances[25,28] and since the C-methyl resonances in zapoterin acetate (**14**) are about the same as those in obacunone (**12**) (δ 1.51, 1.51, 1.45, 1.25, 1.12) a 12α configuration is suggested for zapoterin (**13**).[29]

The mother liquors from the isolation of zapoterin (**13**) gave a further new limonoid. This new limonoid was found in the extracts of seeds from two different seasons. The infrared spectrum showed a hydroxyl band and two different carbonyl bands. The nmr spectrum indicated that it was an obacunone derivative and suggested it might be an obacunol. This was confirmed by the ORD curve which indicated the absence of a keto carbonyl group. Chromic acid oxidation of the new limonoid gave obacunone (**12**). As the product was nonidentical with synthetic 7β-obacunol,

(15) H. Grisebach, "Biosynthetic Patterns in Microorganisms and Higher Plants," John Wiley and Sons, Inc., New York, N. Y., 1967, p 1; H. Grisebach in "Chemistry and Biochemistry of Plant Pigments," T. W. Goodwin, Ed., Academic Press, New York, N. Y., 1965, p 279.

(16) A. Meisels and F. Sondheimer, *J. Amer. Chem. Soc.*, **79**, 6328 (1957).

(17) F. Sondheimer and A. Meisels, *J. Org. Chem.*, **23**, 762 (1958).

(18) R. Johnstone, J. R. Price, and A. R. Todd, *Aust. J. Chem.*, **11**, 562 (1958).

(19) H. C. Beyerman and R. W. Rooda, *Koninkl Ned. Akad. Wetenschap. Proc. Ser. B*, **63**, 432 (1960); *Chem. Abstr.*, **55**, 10488 (1961).

(20) S. Goodwin, A. F. Smith, and E. C. Horning, *J. Amer. Chem. Soc.*, **79**, 2239 (1957).

(21) For a review, see D. L. Dreyer, *Fortschr. Chem. Org. Naturstoffe*, **26**, 190 (1968).

(22) T. Reichstein, *Helv. Chim. Acta*, **15**, 1110 (1932).

(23) D. L. Dreyer, *J. Org. Chem.*, **30**, 749 (1965).

(24) The ir spectrum was identical with that of a sample of zapoterin from the original Syntex work.[4]

(25) For previous nmr data on limonoids, see, D. L. Dreyer, *Tetrahedron*, **21**, 75 (1965). H-2 of obacunone in Table I of this reference should be 358 cps.

(26) Treatment of zapoterin with acetic anhydride–pyridine under conditions reported[4] to give isozapoterin appeared to result in incomplete acetylation as judged by tlc.

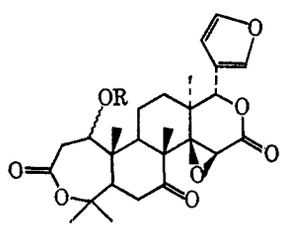
(27) The H-1 resonances in the nmr spectra of **12–15** are very constant. The presence of 11-hydroxy in the zapoterin series should result in a substantial downfield shift of H-1 compared to H-1 in obacunone; *cf.* H-1 resonance in gedunin, cedrelone and anthothecol, J. W. Powell, *J. Chem. Soc., C*, 1794 (1966).

(28) D. A. H. Taylor, *Chem. Commun.*, 500 (1967).

(29) The extreme downfield position of H-12 is accounted for by its position in the deshielding region of the epoxy group.[25]

it must be 7α-obacunol (**17**).[30] 7β-Obacunol is the major product from borohydride reduction of obacunone and by analogy with the borohydride reduction of limonin must have a 7β-hydroxy group.[31] The stereochemistry of 7α-obacunol (**16**) is further shown from the small coupling constants of the H-7 resonance.[25] Direct comparison with synthetic 7α-obacunol provided by Professor T. A. Geissman showed complete identity.

Work-up of the most polar eluents from the column gave deacetylnomilin (**18**).[23] Obacunone (**12**)[32] and nomilin (**19**)[6] have previously been reported from *C. edulis* seeds.



18, R = H
19, R = Ac

The formulation of zapoterin as 12-hydroxyobacunone is of special biogenetic interest. Several publications have emphasized the biogenetic reationship of simaroubolides with limonoids.[33-35] However, instead of the limonol to merolimonol analogy as originally proposed,[33,34] it appears that a reverse aldol or a β-diketone fragmentation as pointed out by Moss[36] is the more reasonable route from limonoids to simaroubolides. This is consistent with the fact that C-12 is an invariant site of oxidation in simaroubolides.[37]

It is a well-established fact that limonoids occur at relatively high concentrations in early season citrus fruits and that the limonin concentration drops off to relatively low levels with advancing maturity.[38] It appears possible that a limonoid fragmentation *via* a 12-keto derivative to simaroubolides may account for the decreasing limonin content in citrus. In spite of careful search using Ehrlich's reagent as a selective detecting reagent, no limonoid metabolites have been found in *Citrus* which can account for the loss of limonin. However, the detection of small amounts of simaroubolides is a much more difficult problem.

(30) F. M. Dean and T. A. Geissman, *J. Org. Chem.*, **23**, 596 (1958); Dean and Geissman named the major product of borohydride reduction of obacunone as α-obacunol and the minor product as β-obacunol. The stereochemistry of the hydroxy group in either was unknown at that time. By analogy with the borohydride reduction of limonin,[31] α-obacunol has the β configuration at C-7. We therefore suggest changing the previous names and designate 7α-obacunol as the isomer with the 7α configuration and 7β-obacunol with the 7β configuration.

(31) A. Melera, K. Schaffner, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, **40**, 1420 (1957); D. Arigoni, D. H. R. Barton, E. J. Corey, O. Jeger, L. Caglioti, Sukh Dev, P. G. Ferrini, E. R. Glazier, A. Melera, S. K. Pradhan, K. Schaffner, S. Sternhell, J. F. Templeton, and S. Tobinaga, *Experientia*, **16**, 41 (1960).

(32) F. Sondheimer, A. Meisels, and F. A. Kincl, *J. Org. Chem.*, **24**, 870 (1959).

(33) D. L. Dreyer, *Experientia*, **20**, 297 (1964).

(34) J. B-son Bredenberg, *Chem. Ind.* (London), 73 (1964).

(35) J. Moron, J. Rondest, and J. Polonsky, *Experientia*, **22**, 511 (1966); J. Moron and J. Polonsky, *Tetrahedron Lett.*, 385 (1968).

(36) G. P. Moss, *Planta Med. Suppl.*, 86 (1966).

(37) For a summary, see J. Polonsky, *ibid.*, 107 (1966).

(38) J. F. Kefford and B. V. Chandler, *Aust. J. Agr. Res.*, **12**, 56 (1961); K. W. Wilson and C. A. Crutchfield, *Agr. Food Chem.*, **16**, 118 (1968); V. P. Maier and G. D. Beverly, *J. Food Sci.*, in press.

Experimental Section[39]

Solvent was removed from the acetone extracts of dried and ground *C. edulis* seeds, collected on the campus of the University of California at Los Angeles and the Los Angeles County Nursery. The residue was chromatographed on alumina. The content of the fractions was monitored by tlc and the spots were detected by viewing under ultraviolet light. Permethylated flavones were detected as bright yellow spots by fuming with hydrogen chloride gas. Limonoids were detected by spraying with Ehrlich's reagent.[23] Oils and waxes were eluted from the column with hexane and discarded. The initial benzene eluents showing fluorescence spots on tlc were first crystallized from MeOH to remove large amounts of β-sitosterol. Benzene (10%) in hexane eluted the 5-methoxy-8-geranyloxypsoralen–phellopterin complex: mp 65–68°, from ethyl acetate–hexane; nmr ($CDCl_3$), δ 8.06 (d, J = 10 Hz, H-4), 7.62 (d, J = 2 Hz, H-7), 7.00 (d, J = 2 Hz, H-6), 6.20 (d, J = 10 Hz, H-3), 5.60 (t, J = 7 Hz, H-2′), 4.84 (d, J = 7 Hz, H-1′), 4.16 (s, methoxy), 2.02, 1.98 (H-4′ and H-5′), 1.70, 1.68, 1.65, 1.63 (C-methyls). The H-6′ vinyl resonance is partly obscured by H-1′.

Anal. Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 69.9; H, 6.05.

Further elution of the column with increasing amounts of benzene in hexane gave fractions which yielded phellopterin (**1**): mp 99–100°, from EtOAc–hexane; λ_{max}^{EtOH} 219 mμ, 238, 244, 265, 310; nmr ($CDCl_3$), δ 8.07 (d, J = 10 Hz, H-4), 7.62 (d, J = 2 Hz, H-7), 6.98 (d, J = 2 Hz, H-6), 6.22 (d, J = 10 Hz, H-3), 5.60 (t, J = 7 Hz, vinyl), 4.81 (d, J = 7 Hz, α-methylene), 4.15 (methoxy), 1.72 (C-methyls).

Further elution with 80–90% benzene in hexane gave fractions containing mostly zapotin, crystallized from ethyl acetate–hexane or methanol. All mother liquors still showing evidence for permethylated flavanoids were combined and rechromatographed. Work-up of the fractions gave 2′,5,6-trimethoxyflavone (**4**), mp 121–125°, from EtOAc–hexane or ether–hexane. Ultraviolet and infrared spectra were identical with those of an authentic sample:[7] λ_{max}^{EtOH} 234 mμ (ε 17,000), 269 (18,500), 324 (12,100).

Further crystallization of the mother liquors from EtOAc–hexane or ether–hexane gave the casimiroa flavone: mp 118–120°; λ_{max}^{EtOH} 230, 273, 304 mμ; nmr ($CDCl_3$), δ 7.45 (multiplet center, H-2′, H-5′, and H-6′), 7.31 (s, H-7 and H-8), 7.04 (multiplet center, H-4′), 6.64 (s, H-3), 3.98, 3.92, 3.87, 3.85 (methoxy); (benzene-d_6), δ 7.40–6.55 (complex multiplet), 6.95, 6.76 (AB doublet, J = 9 Hz, H-6 and H-7), 4.09, 3.47, 3.44 (methoxys); mass spectrum m/e 103 (18), 131 (21), 132 (10), 137 (15), 149.5 (10), 163 (12), 175 (12), 190 (12), 200 (15), 219 (15), 254 (6), 267 (10), 269 (12), 281 (10), 282 (10), 283 (12), 297 (100), 312 (64), 327 (14), 342 (9).

Anal. Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 68.4; H, 5.26.

The flavone mixture was subsequently separated into **8** and **9** by vpc on 5 ft × 0.125 in. 5% SE-30 on Chromosorb W 60–80 mesh at 230°. The trimethoxy derivative **8** had a retention time of 15 min, and the tetramethoxyflavone **9** has a retention time of 33 min under these conditions.

Fractions eluted from the column with increasing amounts of chloroform in benzene gave casimiroin,[16] followed by eduline. Small amounts of 1-methyl-2-phenyl-4-quinolone[20,40] and eduline were recovered by work-up of the eduline mother liquors.

1-Methyl-2-phenyl-4-quinolone had the following properties: λ_{max}^{EtOH} 211 mμ, 250, ~326, 336; $\lambda_{max}^{EtOH\text{-}HCl}$ 233 mμ, 250, 310; nmr ($CDCl_3$), δ 8.53 (J = 2 Hz, H-5), 7.48 (m, aromatic), 6.28 (s, H-3), 3.60 (s, N-methyl).

Eduline had the following properties: nmr ($CDCl_3$), δ 7.89 (d, J = 3 Hz, H-1), 7.47 (m, aromatic), 6.25 (s, H-3), 3.93 (s, methoxy), 3.60 (N-methyl); edulein mp 194–196° (EtOAc); λ_{max}^{EtOH} ~235 mμ, 251, 259, 316, 326; $\lambda_{max}^{EtOH\text{-}HCl}$ ~232 mμ, 252, 309, 331; nmr ($CDCl_3$), δ 8.43 (d, J = 9 Hz, H-5), 7.50 (aromatic), 6.25 (s, H-3), 3.95 (methoxy), 3.55 (N-methyl). The ir spectrum was identical with that published by Price and coworkers.[18]

Removal of solvent from the chloroform eluents and crystallization of the residue from methanol or methanol–ether gave zapoterin, recrystallized from methanol–acetonitrile for analysis:

(39) Nmr spectra were taken at 60 MHz. The relative area of the peaks were consistent with the assignments.

(40) B. Witkop and J. B. Patrick, *J. Amer. Chem. Soc.*, **74**, 3855 (1952).

mp 269–271°; R_f 1.3 that of limonin on tlc; ν (Nujol) 3370 (hydroxyl), 1758, 1720, 1668 (carbonyl) 1627 (double bond), 1511, 833 (β-substituted furan) cm^{-1}; $\lambda_{max}^{CH_3CN}$ 209 mμ (ε 23,000); ORD (c 0.16), $[\alpha]_{600}$ −36°, $[\alpha]_{310}$ −1150° $[\alpha]_{303}$ −1070°, $[\alpha]_{301}$ −1090°, $[\alpha]_{260}$ +840° (sh), $[\alpha]_{241}$ +1470°; nmr (CF_3COOH), δ 7.50 (J = 2 Hz, α-furan), 7.03 (d, J = 13 Hz, H-1), 6.47 (t, J = 2 Hz, β-furan), 6.19 (d, J = 13 Hz, H-2), 5.82 (s, H-17), 4.99 (t, $W_{1/2}$ = 11 Hz, H-12), 4.03 (s, H-15), 1.90, 1.68, 1.68, 1.55, 1.18 (C-methyls).

Anal. Calcd for $C_{26}H_{30}O_8$: C, 66.37; H, 6.43. Found: C, 66.3; H, 6.36.

Fractions eluted from the column with chloroform and following the zapoterin gave 7α-obacunol (**17**): mp 242–245°, from ethanol; ν (Nujol) 1750, 1684 (carbonyl), 1627 (double bond), 1512, 883 (β-substituted furan) cm^{-1}; ORD in dioxane (c 0.09), $[\alpha]_{600}$ +91°, $[\alpha]_{332}$ +250°, $[\alpha]_{281}$ −795° $[\alpha]_{268}$ −230° (sh), $[\alpha]_{250}$ +1250° (last reading); nmr ($CDCl_3$), δ 7.49 (d, J = 1 Hz, α-furan), 6.57 (d, J = 12 Hz, H-1), 6.42 (t, J = 1 Hz, β-furan), 5.89 (d, J = 12 Hz, H-2), 5.64 (s, H-17), 3.90 (s, H-15), 3.52 (t, J = 2 Hz, H-7), 1.43, 1.30, 1.25, 1.25, 1.05 (C-methyls).

Anal. Calcd for $C_{26}H_{32}O_7$: C, 68.40; H, 7.07. Found: C, 68.3; H, 7.03.

Further elution of the column with chloroform and chloroform–acetone gave fractions which after work-up yielded deacetylnomilin (**18**), mp 264–265° dec, from methanol. Its infrared spectrum was superimposable on that of an authentic sample isolated from *Citrus*.[23]

Acid Hydrolysis of 2.—A solution of 20 mg of the coumarin in 5 ml of glacial AcOH and 1 drop of concentrated HCl was heated on a steam bath for 45 min. Cooling, dilution with water, and extraction with EtOAc gave 5-methoxy-8-hydroxypsoralen (**3**), identical with a sample isolated from *C. edulis* by published procedures:[4] λ_{max}^{EtOH} 224 mμ, 274, 317.

Preparation of 2.—A solution of 100 mg of 5-methoxy-8-hydroxypsoralen and 100 mg of geranyl bromide[8] in acetone was refluxed overnight with anhydrous potassium carbonate. Cooling, filtration, and removal of solvent gave an oil which was filtered through a short column of alumina with benzene. The product (**2**) could only be obtained crystalline at Dry Ice temperature: nmr ($CDCl_3$), δ 7.90 (d, J = 10 Hz, H-4), 7.53 (d, J = 2 Hz, H-7), 6.95 (d, J = 2 Hz, H-6), 5.90 (d, J = 10 Hz, H-3), 5.52 (t, J = 7 Hz, H-2′), 4.85 (coupling obscured, H-6′), 4.77 (d, J = 7 Hz, H-1′), 4.01 (methoxy), 2.00, 1.96 (H-4′ and H-5′), 1.70, 1.63, 1.55 (C-methyls).

Demethylation of the Casimiroa Flavone.—A solution of 100 mg of **6** in 30 ml of 20% hydrochloric acid was refluxed for 25 min. The flavone quickly went into solution, and the product began to crystallize out after 15 min. The solution was cooled and the yellow 5-demethyl derivative was collected by filtration and recrystallized from methanol: mp 140°; green ferric chloride; λ_{max}^{EtOH} 270 mμ, ∼320; $\lambda_{max}^{EtOH\text{-}AlCl_3}$ mμ, 302 ∼330; nmr ($CDCl_3$), δ 7.48–6.92 (complex multiplet, B-ring protons), 7.25, 6.92 (AB doublet, J = 9 Hz, H-7 and H-8), 6.62 (s, H-3), 3.94, 3.89, 3.85 (methoxy); (benzene), δ 3.60, 3.42 (methoxy).

5-Acetoxy Derivative.—The 5-demethylflavone was acetylated on a 30-mg scale by heating with acetic anhydride–pyridine for 1 hr on a steam bath: mp 134–135°, from methanol; λ_{max}^{EtOH} ∼218, 273, 304, ∼330 mμ; nmr ($CDCl_3$), δ 7.45–6.90 (complex multiplet, B-ring protons), 7.39 (s, H-7 and H-8), 6.61 (s, H-3), 3.88, 3.85, 3.84 (methoxys), 2.47 (acetoxy); (benzene-d_6), δ 7.25–6.8 (complex multiplet, B-ring protons), 6.99, 6.75 (AB doublet, J = 9 Hz, H-7 and H-8), 6.54 (s, H-3), 3.38, 3.38, 3.35 (methoxy), 2.38 (acetoxy).

5-Demethylzapotin (**6**) had the following nmr data ($CDCl_3$): δ 7.44 (t, J = 8 Hz, H-4′), 7.25 6.90 (AB doublet, J = 9 Hz, H-7 and H-8), 6.65 (d, J = 8 Hz, H-3′ and H-5′), 6.32 (s, H-3), 3.95 3.80, 3.80 (methoxys).

5-Demethylzapotin acetate (**7**) showed the following nmr data ($CDCl_3$): δ 7.42 (t, J = 8 Hz, H-4′), 7.36 (s, H-7 and H-8), 6.64 (d, J = 8 Hz, H-3′ and H-5′), 6.24 (s, H-3), 3.88, 3.80, 3.80 (methoxys), 2.48 (acetoxy); (benzene), δ 3.27, 3.20, 3.20 (methoxys), 2.35 (acetoxy).

Base Fusion of Casimiroa Favone.—A 30-mg sample of **6** was refluxed with 20% aqueous NaOH for 1 hr. The solution was cooled, extracted with ether, and acidified. The acidic solution was extracted six times with ether. The ether extracts were extracted three times with 5% sodium bicarbonate. The bicarbonate extracts were acidified and extracted six times with ether. Removal of solvent from the ether extracts and sublimation of the residue gave 3,5-dimethoxybenzoic acid after recrystallization from benzene–hexane. The product was identical in all respects with an authentic sample. 3-Methoxybenzoic acid could not be obtained pure from the mother liquors by crystallization.

Hydrogen Peroxide Oxidation of the Casimiroa Flavone.—A solution of 40 mg of the natural flavone mixture in 1:1 EtOH–10% aqueous NaOH was treated with 30% hydrogen peroxide by methods described previously.[41] The reaction was worked up in the same manner as that from the base hydrolysis. Again only the presence of 3,5-dimethoxybenzoic acid could be clearly established in the products by crystallization.

3′,5,6-Trimethoxyflavone (**8**).—A solution of 1 g of 2-hydroxy-5,6-dimethoxyacetophenone[42] and 1 g of 3,5-dimethoxybenzoyl chloride in acetone was refluxed 12 hr over anhydrous potassium carbonate. The cooled solution was filtered, and the solvent was removed from the filtrates. The residue was refluxed 6 hr with AcOH–AcONa. The acetic acid solution was poured into water and extracted with chloroform. The chloroform extracts were filtered through a short column of alumina. Solvent was removed from the eluents and the residue crystallized from ether–EtOAc: mp 126–127°; λ_{max}^{EtOH} ∼209 mμ, 234 (ε 16,800), 272 (25,200), 302 (13,000), ∼317 (12,000); nmr ($CDCl_3$), δ 7.42 (m, H-2′, H-5′, and H-6′), 7.30 (s, H-7 and H-8), 7.03 (m, H-4′), 6.63 (s, H-3), 3.99, 3.92, 3.87 (methoxy); (benzene), δ 4.05, 3.50, 3.47 (methoxy).

Anal. Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.4; H, 5.16.

3′,5,5′,6-Tetramethoxyflavone (**9**).—This product was prepared from 2-hydroxy-5,6-dimethoxyacetophenone[42] and 3,5-dimethoxybenzoyl chloride in the same manner as the trimethoxyflavone **8**: mp 135–137° (ether–EtOAc); λ_{max}^{EtOH} ∼229 mμ, 218 (ε 21,700), 306 (17,000); nmr ($CDCl_3$), δ 7.40 (s, H-7 and H-8), 7.00 (d, J = 2 Hz, H-2′ and H-6′), 6.65 (s, H-3), 6.60 (t, J = 2 Hz, H-4′), 3.99, 3.94, 3.87, 3.87 (methoxy); (benzene), δ 4.03, 3.45, 3.42, 3.42 (methoxy).

Anal. Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.6; H, 5.21.

Zapoterin Acetate (**14**).—Zapoterin was acetylated by refluxing with acetic anhydride–pyridine for 1.5 hr: mp 277–280°, from EtOAc; ν (Nujol) 1754 1739, 1690 (carbonyl), 1639 (double bond), 1511, 882 (β-substituted furan) cm^{-1} (Nujol); nmr ($CDCl_3$), δ 7.44 (d, J = 2 Hz, α-furan), 6.62 (d, J = 12 Hz H-1), 6.36 (t, J = 2 Hz, β-furan), 6.00 (d, J = 12 Hz, H-2) 5.74 (t, J = 2 Hz, H-12), 5.64 (s, H-17), 3.75 (s, H-15), 2.18 (acetoxy), 1.53, 1.52, 1.47, 1.27, 1.10.

Anal. Calcd for $C_{28}H_{32}O_9$: C, 65.61; H, 6.29. Found: C, 65.5; H, 6.32.

Zapoterone (**15**).—Jones reagent was slowly added dropwise to a solution of zapoterin in acetone with ice bath cooling. After 5 min the solution was allowed to warm to room temperature. Further Jones reagent was added as necessary to keep the solution a chromic acid color. After 20 min at room temperature, the solution was diluted with water and extracted with chloroform. The chloroform extracts were dried and filtered through a short column of alumina, and the solvent was removed. The residue was crystallized from ether–methanol to give zapoterone: mp 249–251°, from EtOAc; ν (Nujol) 1760, 1739, 1696 (carbonyl), 1645 (double bond), 1512, 884 (β-substituted furan) cm^{-1}; nmr ($CDCl_3$), δ 7.47 (d, J = 1 Hz, α-furan), 6.64 (d, J = 12 Hz, H-1), 6.40 (t, J = 1 Hz, β-furan), 5.85 (d, J = 12 Hz, H-2), 5.54 (s, H-17), 3.98 (s, H-15), 1.85, 1.52, 1.47, 1.35, 1.30 (C-methyls).

Anal. Calcd for $C_{26}H_{28}O_8$: C, 66.65; H, 6.02. Found: C, 66.2; H, 6.05.

Oxidation of 7α-Obacunol.—A solution of 7α-obacunol in acetone was oxidized with Jones reagent as described for zapoterone. The product was recrystallized from methanol to give obacunone, mp 226–227.5°. Its infrared spectrum was superimposable on that of an authentic sample.

Registry No.—**1**, 2543-94-4; **2**, 17182-52-4; **6**, 14813-20-8; **7**, 17182-54-6; **8**, 17182-55-7; **9**, 17182-56-8; **13**, 17182-57-9; **14**, 17182-58-0; **15**, 17188-76-0; **17**, 17182-59-1; 1-methyl-2-phenyl-4-quinolone, 17182-60-4; eduline, 6878-08-6; edulein, 483-51-2.

(41) See, for example, W. D. Ollis, C. A. Rhodes, and I. O. Sutherland, *Tetrahedron*, **23**, 4741 (1967).

(42) W. Baker, *J. Chem. Soc.*, 956 (1939).

Acknowledgments.—The author is indebted to Professor T. A. Geissman, University of California at Los Angeles, for a synthetic sample of 7α-obacunol, to Professor H. Mitsuhashi, University of Hokkaido, Japan, for the ORD data, to L. M. White for the analytical data, to Allen Singer for the vpc results, and to Dr. R. M. Horowitz for helpful discussions.

5-Amino-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose[1]

U. G. NAYAK AND ROY L. WHISTLER

Department of Biochemistry, Purdue University, Lafayette, Indiana 47907

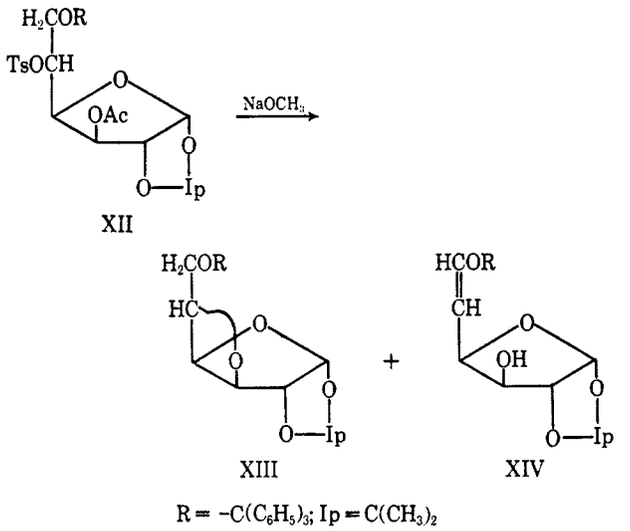
Received March 1, 1968

Two convenient routes for the synthesis of 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose are provided by (1) nucleophilic displacement of the *p*-tolylsulfonyloxy group in 3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-(*p*-tolylsulfonyl)-β-L-idofuranose with azide anion, and (2) nucleophilic opening of the oxetan ring of 3,5-anhydro-1,2-*O*-isopropylidene-β-L-idofuranose with azide anion followed by hydrogenation.

This laboratory has been interested in the synthesis of 5-thio- and 5-aminofuranose sugars[2-9] which can be cyclized under acetolysis conditions to the pyranose sugars in which the ring heteroatom is either sulfur or nitrogen. The SN2 displacement of the sulfonyloxy group in the appropriate 5-*O*-*p*-tolylsulfonyl derivative with nucleophiles or nucleophilic opening of the oxetan ring offers convenient routes for the placement of sulfur or nitrogen at C-5. Both of these synthetic methods for introducing an amino group at C-5 of a D-glucofuranose derivative are described.

In the first procedure the *p*-tolylsulfonyloxy group in 3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-(*p*-tolylsulfonyl)-β-L-idofuranose[9] (I) is displaced with azide anion in refluxing *N*,*N*-dimethylformamide (DMF) to give a 65% yield of 5-azido-3,6-di-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (II). The infrared spectrum of II in Nujol shows a strong absorption at 2150 cm^{-1}, characteristic of an azide group. The integrated nmr spectrum of compound II in $CDCl_3$ shows a total of 27 protons, ten of which are aromatic (τ 2.5) and six of which represent the isopropylidene methyl protons at 8.53 and 8.7. Other definitive signals in the nmr spectrum are for one anomeric proton as a doublet centered at τ 4.1 (J = 3.5 Hz) and four methylene protons of the benzyl groups at 5.36 and 5.42. It is interesting to note that even in this displacement reaction an 8% yield of the benzyl vinyl ether III (R_f 0.25 in solvent A) is formed by a β-proton elimination, a reaction not seen in other compounds.[10] This observation supports the view of Buchanan and Oakes,[11] who have emphasized that the formation of the olefin XIV and the 3,5-anhydro compound XIII are two independent simultaneous reactions occurring when 3-*O*-acetyl-1,2-*O*-isopropylidene-5-*O*-(*p*-tolylsulfonyl)-6-*O*-trityl-α-D-glucofuranose XII is refluxed with sodium methoxide in methanol (Scheme I). The infrared spectrum of III shows a strong absorption at 1650 cm^{-1} (benzyl vinyl ether). The integrated nmr spectrum of III in $CDCl_3$ shows a total of 26 protons, ten of which are aromatic (τ 2.67) and six are for the isopropylidene methyl protons (8.5 and 8.7). Other signals are for an anomeric proton as a doublet centered at τ 4.06 (J = 3.5 Hz), four methylene protons of the benzyl groups at 5.2 and 5.43, and the two vinyl protons at C-6 and C-5 each of unit area, shown as doublets centered at 3.18 (J = 12 Hz) and 4.9 (J = 12 Hz), respectively. The large coupling constant for the vinyl proton signals suggests that the protons are *trans* to each other and that compound III is 3,6-di-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose.

SCHEME I



In addition to the olefin III having an R_f value of 0.25 in solvent A, another olefin with an R_f value of 0.39, suggestive of structure IV or V, is isolated in 6% yield. The nmr spectrum of olefin IV or V in $CDCl_3$ shows a perfect integration for 26 protons, ten of which are aromatic at τ 2.67, six are for the methyls of the isopropylidene and 8.58 and 8.65, and one is for the anomeric C-1 as a doublet centered at 3.88 (J = 3.5 Hz). The other nine protons are spread between τ 5 and 5.83. However, it is significant that there are no vinyl proton signals shown in the usual olefinic proton range of τ 3–5 (except the anomeric proton). Also the methyl proton

(1) This work was supported by the Agricultural Research Service, U. S. Department of Agriculture, Grant No. 12-14-100-7662 (71) administered by the Northern Regional Laboratory, Peoria, Ill., Journal Paper No. 3307 of the Purdue Agricultural Experiment Station, Lafayette, Ind.

(2) M. S. Feather and R. L. Whistler, *Tetrahedron Lett.*, No. 15, 667 (1962).
(3) R. M. Rowell and R. L. Whistler, *J. Org. Chem.*, **31**, 1514 (1966).
(4) D. L. Ingles and R. L. Whistler, *ibid.*, **27**, 3896 (1962).
(5) R. L. Whistler, M. S. Feather, and D. L. Ingles, *J. Amer. Chem. Soc.*, **84**, 122 (1962).
(6) R. L. Whistler and R. M. Rowell, *J. Org. Chem.*, **29**, 1259 (1964).
(7) R. L. Whislter, W. E. Dick, T. R. Ingle, R. M. Rowell, and B. Urbas, *ibid.*, **29**, 3723 (1964).
(8) R. E. Gramera, R. M. Bruce, S. Hirase, and R. L. Whistler, *ibid.*, **28**, 1401 (1963).
(9) R. L. Whistler and R. E. Gramera, *ibid.*, **29**, 2609 (1964).
(10) R. E. Gramera, T. R. Ingle, and R. L. Whistler, *ibid.*, **29**, 1083 (1964).
(11) J. G. Buchanan and E. M. Oakes, *Carbohyd. Res.*, **1**, 242 (1965).