[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY, AND VARIAN ASSOCIATES]

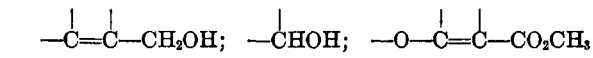
Terpenoids. XLVII.[1] The Structure of Genipin[2]

CARL DJERASSI, T. NAKANO, A. N. JAMES, L. H. ZALKOW, E. J. EISENBRAUN, AND J. N. SHOOLERY

Received July 18, 1960

Extensive degradation experiments and NMR measurements are reported which lead to expression I for genipin. Its absolute configuration has been established by degradation to the dibasic acid XIIIa, which was shown to be the antipode of one of the nepetic acids derivable from nepetalactone (XI). The close biogenetic relationship of genipin to aucubin, plumieride, and other monoterpenoids is emphasized. Attention is also called to the behavior of some genipin degradation products towards lithium aluminum hydride resulting in cleavage of the dihydropyran ring.

In an earlier article,[3] there was reported the isolation of the active principle of *Genipa americana* L. as well as the characterization of its functional groups. The substance, named genipin, corresponded in terms of empirical formula to $C_{11}H_{14}O_5$ and its five oxygen functions could be defined as follows:



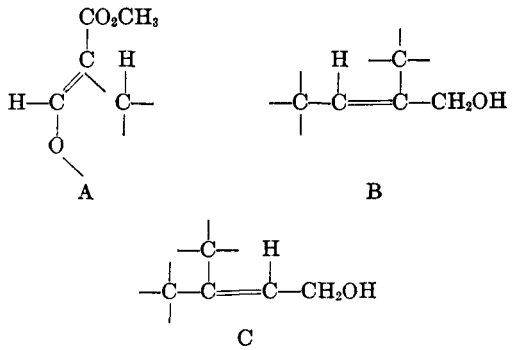
Furthermore, it was noted that genipin had to be bicyclic and that the ether oxygen atom was involved in one of these rings. We should now like to present degradative and spectroscopic evidence which establishes unambiguously the structure and absolute configuration of this substance.

The rather small total number of hydrogen atoms coupled with the relative abundance of oxygen atoms or double bonds indicated that NMR measurements[4] might be very instructive and this has indeed proved to be the case. We shall, therefore, present NMR spectroscopic data concurrently with the most important chemical degradation experiments.

In order to simplify comparison with data obtained at other field strengths, it is proposed that the positions of single peaks and centers of multiplets (see for instance $\delta = 4.81$ in Fig. 1) be expressed in dimensionless "chemical shift" units, $\delta^{int}_{SiMe_4}$, defined as follows:

$\delta^{int}_{Si(CH_3)_4} = 10^6\ (\nu - \nu Si(CH_3)_4)/\nu Si(CH_3)_4$ p.p.m., which for the presently used 60 megacycle instrument reduces to $\delta^{int}_{Si(CH_3)_4} = \frac{\text{c.p.s.}}{60} \times 10^6$ p.p.m. Although this definition leads to a chemical shift scale which differs from the one proposed by Tiers[4c,5] both in direction and in the position of the zero, the present authors feel that it offers an advantage in that the directly measured "working units" of peak position, *i.e.*, c.p.s., can be readily converted by simple division to the field-independent "reporting units," *i.e.*, p.p.m. Furthermore, the assignment of the position of the internal reference compound, tetramethylsilane ($Si(CH_3)_4$), as zero both on the frequency and p.p.m. scales for all instruments regardless of frequency appears to be the least arbitrary choice possible. In consequence of this choice, increasing δ corresponds to decreased shielding.

For the sake of simplicity, the entire discussion will be presented in terms of the eventually established structure I for genipin.[6] Its NMR spectrum, reproduced in Fig. 1, confirms as well as amplifies the characterization of the functional groups made earlier.[3] Diagnostically, the most significant peaks proved to be the ones at $\delta = 7.52$, 5.86, 4.81 (doublet at 293 and 284 c.p.s.), 4.29 and 3.73. The 7.52 peak is assigned to the conjugated olefinic proton (attached to C-3) and the unusually large shift of this peak confirms the location of the oxygen atom as adjacent to it. A very slight doubling of this peak, observable in some spectra of genipin and its transformation products, is characteristic of spin coupling through four bonds, one of which is the double bond, and suggests that the partial structure A is present, the hydrogen atoms of the carbomethoxy group being responsible for the peak at $\delta = 3.73$.



A B C

(1) Paper XLVI, T. Nakano, and C. Djerassi, *J. Org. Chem.*, **26**, 167 (1961).

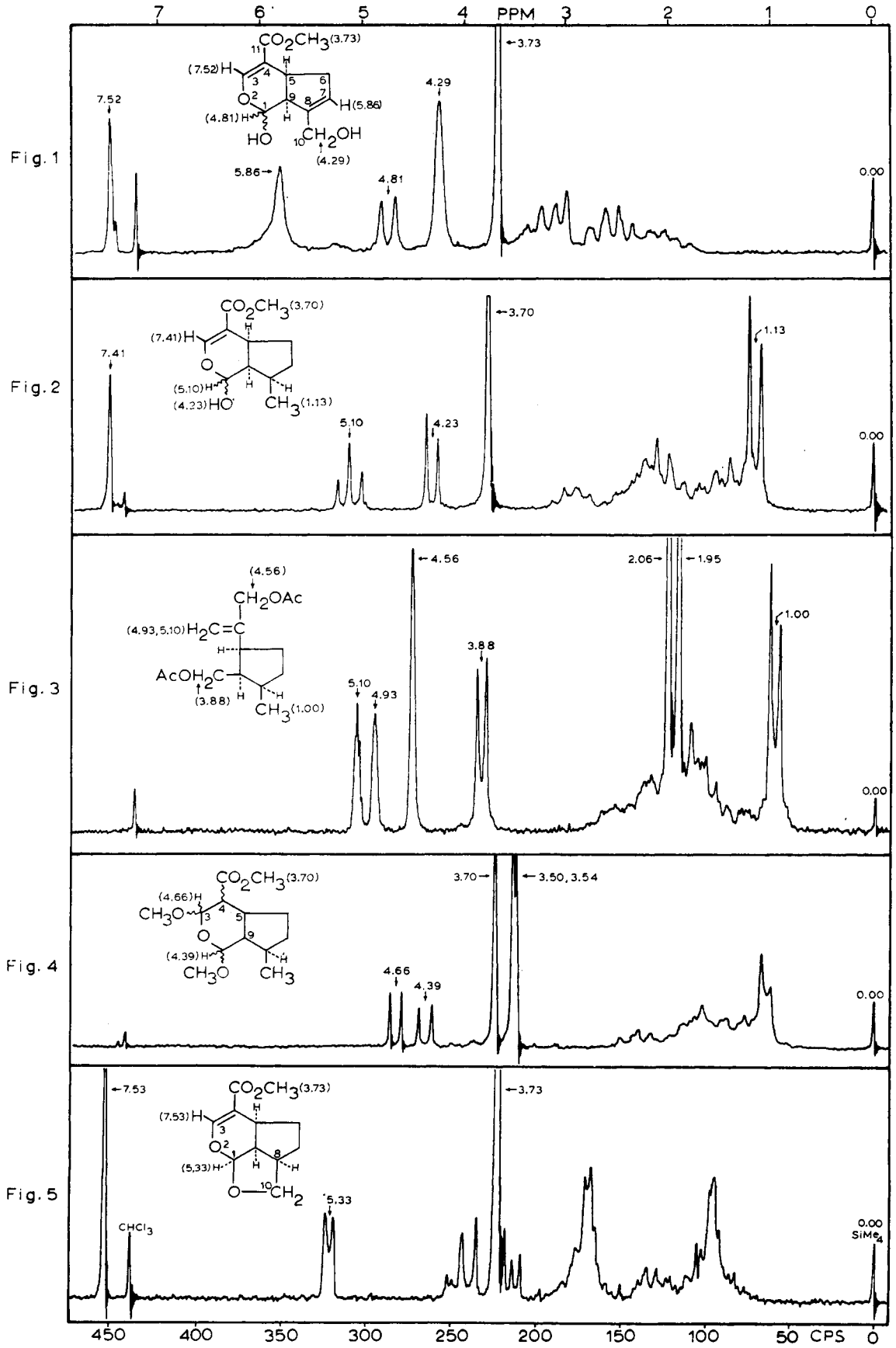
(2) The investigations at Stanford University and Wayne State University were supported by the National Heart Institute (grants No. H-5048 and H-2574) of the National Institutes of Health, U. S. Public Health Service.

(3) C. Djerassi, J. D. Gray, and F. Kincl, *J. Org. Chem.*, **25**, 2174 (1960).

(4) See (a) J. A. Pople, W. G. Schneider, and H. J. Bernstein, *High-resolution Nuclear Magnetic Resonance*, McGraw-Hill, New York, 1959; (b) J. D. Roberts, *Nuclear Magnetic Resonance. Applications to Organic Chemistry*, McGraw-Hill, New York, 1959; (c) L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon Press, London, 1959.

(5) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958). The Tiers τ scale can be readily interconverted into the presently proposed scale by the relationship $\tau = 10 - \delta^{int}_{Si(CH_3)_4}$.

(6) We are employing a numbering system which coincides with that selected for plumieride (O. Halpern and H. Schmid, *Helv. Chim. Acta*, **41**, 1109 (1959)).


7 6 5 4 PPM 3 2 1 0
Fig. 1
7.52
5.86
4.81
4.29
3.73
0.00
Fig. 2
7.41
5.10
4.23
3.70
1.13
0.00
Fig. 3
4.56
5.10
4.93
3.88
2.06
1.95
1.00
0.00
Fig. 4
4.66
4.39
3.70
3.50, 3.54
0.00
Fig. 5
7.53
CHCl3
5.33
3.73
0.00
SiMe4
450 400 350 300 250 200 150 100 50 CPS 0

The presence of an enol ether grouping conjugated with the carbomethoxy function was already indicated earlier[3] by the chemical and especially ultraviolet spectroscopic evidence. The ultraviolet absorption maximum at 240 mμ is much more compatible with partial structure A than one, in which the carbon atom β to the carbomethoxy group is substituted, since the maximum should then be displaced to approximately 248 mμ.[6,7] The NMR peak at $\delta = 5.86$ can be attributed to a single proton on a double bond, thus enabling us to place the allylic primary hydroxyl group, known[3] to be present on the basis of hydrogenation experiments and trityl ether formation, into partial structures B or C. The peak at $\delta = 4.29$ is due to the hydrogen atoms of the methylene group (C-10 to which the primary hydroxyl group is attached. Structure C is less likely because there exists no spin coupling of the anticipated magnitude between the olefinic proton and the two adjacent ones.

The doublet (293 and 284 c.p.s.) at $\delta = 4.81$ is caused by the proton adjacent to the secondary hydroxyl group (C-1) and is shifted to 5.88 when genipin is transformed into its diacetate;[3] similarly, the peak at $\delta = 4.29$ is moved to 4.65. Such shifts are typical of protons adjacent to hydroxyl groups and thus confirm the assignments. Of particular consequence is the 9 c.p.s. spacing of the doublet, since this is characteristic[8] of spin-coupling between two axial protons on adjacent carbon atoms of a six-membered ring. As will be noted below, this represents the most important evidence for the equatorial orientation of the secondary hydroxyl function of genipin.

The remaining peaks in the NMR spectrum (Fig. 1) of genipin (I) are complex due to spin-spin coupling between protons which have small differences in their chemical shifts. All signals have sufficiently large δ values to permit the conclusion that *no protons are attached to carbon atoms which are not adjacent to either an oxygen atom or a doubly bonded carbon atom.*

In our first paper[3] it was pointed out that catalytic hydrogenation of genipin can lead to a fairly complex mixture due to hydrogenation of one or both double bonds as well as hydrogenolysis. Since one of the complicating aspects of the chemistry of genipin is its great lability to acid or base, it was desirable to remove some of the oxygen functions in the hope that a transformation product would be obtained which would lend itself to more convenient chemical manipulations. Therefore, we returned to a careful examination of the catalytic hydrogenation of genipin and as noted in the Experimental section, depending upon the conditions, five pure products (IIa, XXIVa, XXVI, XXVII, XXXIII) could be isolated and their constitutions determined. The single most important hydrogenation product was 10-deoxy-7,8-dihydrogenipin (IIa)[6] and its structure proof will be presented first since it led directly to the structure of genipin itself.

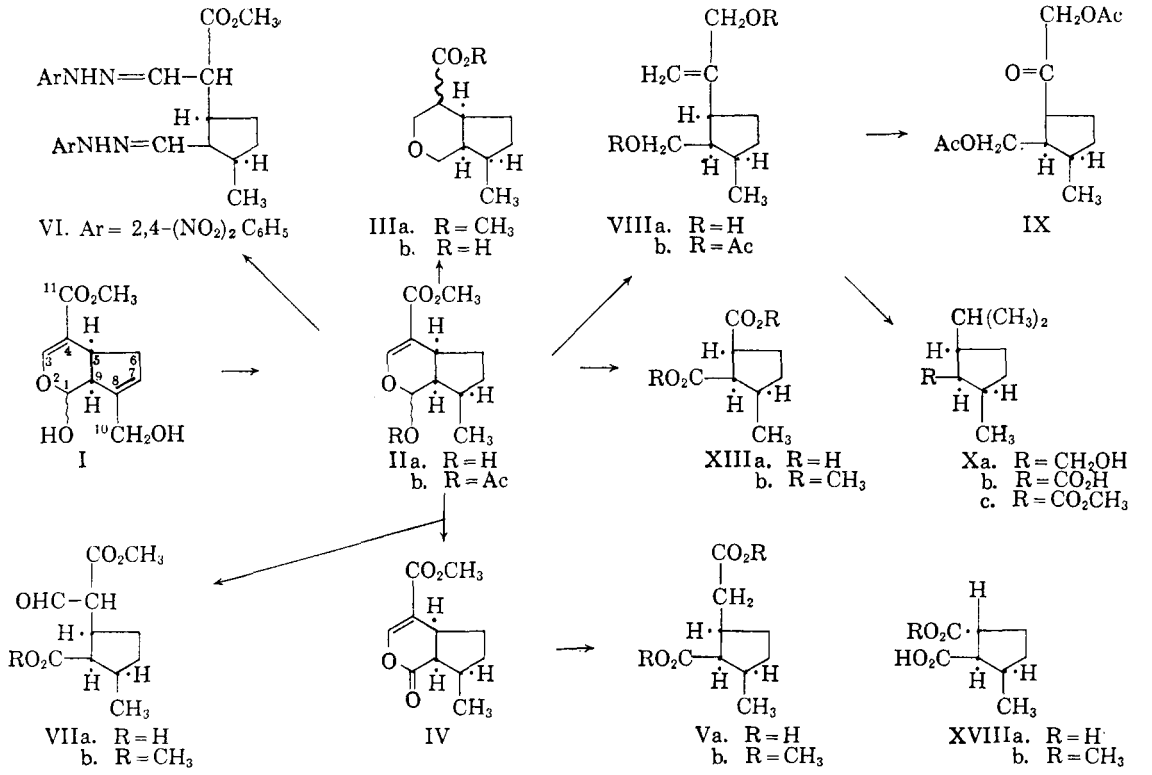
10-Deoxy-7,8-dihydrogenipin (IIa). Of the five hydrogenation products of genipin (I), only one, 10-deoxy-7,8-dihydrogenipin (IIa) is produced both with palladium charcoal in methanol solution or with platinum oxide in acetic acid. From a preparative standpoint (see Experimental), the latter method was preferable and a pure product could be isolated in over 30% yield. The empirical formula $C_{11}H_{16}O_4$ of the crystalline substance (IIa) m.p. 81.5–83°, $[\alpha]_D$ −2.4°, when contrasted with that of genipin (I) ($C_{11}H_{14}O_5$), showed that reduction of one double bond and hydrogenolytic loss of one hydroxyl group had occurred. Accordingly, only a monoacetate (IIb) was formed upon acetylation. Genipin (I) does not possess a C-methyl function, while the presence of one such grouping could be demonstrated in IIa by Kuhn-Roth oxidation. It follows, therefore, that the allylic primary hydroxyl group had been lost and since the ultraviolet absorption spectrum (λ_{max} 240 mμ, log ε 4.06) was essentially identical with that of genipin, the enol ether double bond was intact and the isolated double bond had been reduced. This conclusion was confirmed by the infrared and especially the NMR spectrum (Fig. 2). The latter had lost the peak at 5.86, due to the proton on the isolated double bond of genipin (Fig. 1), but still retained the peaks at $\delta = 7.41$ (single proton on enol ether double bond) and 3.70 (carbomethoxy group) showing that partial structure A is present in unaltered form in IIa.

A doublet (64 and 71 c.p.s.) at $\delta = 1.13$ is characteristic of a methyl resonance split by spin coupling to *one* proton on the adjacent carbon atom. It can be concluded, therefore, that partial structure B rather than C has given rise to this structural fragment by hydrogenation with hydrogenolysis.

A triplet is found at $\delta = 5.10$, which changes to a doublet upon addition of a trace of hydrogen chloride gas to the sample. A doublet at $\delta = 4.23$ undergoes a similar collapse to a singlet and shifts slightly to 4.31 upon addition of hydrogen chloride, showing that it arises from a hydroxyl group which couples its spin to the adjacent CH group. The *downfield* shift of 0.29 p.p.m. of the CH group (C-1) adjacent to this hydroxyl function in going from genipin (I) to its reduction product IIa is a strong indication that the secondary hydroxyl group is not adjacent to the unconjugated double bond (partial structure B) of genipin (I), since it would have been expected to shift *upfield* upon removal of the unshielding effect of an adjacent unsaturated carbon atom. The 7 c.p.s. spin coupling of the C-1 proton at $\delta = 5.10$ suggests that this proton is

(7) F. E. Bader, *Helv. Chim. Acta*, 36, 215 (1953).

(8) See for instance Chap. 14 in Ref. 4a and p. 86 in Ref. 4c.



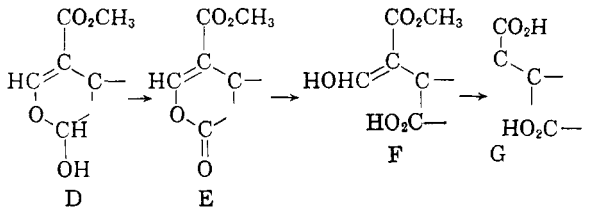
slightly less axial in 10-deoxy-7,8-dihydrogenipin (IIa) than it was in genipin (I). This would also account for the downfield shift of 0.29 p.p.m. After addition of hydrogen chloride gas to promote hydroxyl exchange, it was observed that the 5.10 peak slowly (over a period of a few minutes) acquired a neighbor doublet at $\delta = 5.51$ with a 3 c.p.s. spin coupling. After removal of the solvent and the hydrogen chloride by vacuum distillation, re-examination in fresh, acid-free deuterochloroform disclosed two hydroxyl resonances as well as the two CH resonances at 5.10 and 5.51. These results clearly show that a trace of strong acid catalyses the isomerization of some of the secondary hydroxyl group from the equatorial to the axial orientation, resulting in an equilibrium mixture which contains about 75% equatorial and 25% axial hydroxyl components.

When 10-deoxy-7,8-dihydrogenipin (IIa) was hydrogenated further with Raney nickel catalyst at elevated temperature and pressure, reduction of the conjugated double bond was accompanied by loss of the secondary hydroxyl group. The formation of 1,10-bisdeoxy-3,4,7,8-tetrahydrogenipin (IIIa) implies that the secondary hydroxyl group is also potentially labile and its behavior towards oxidizing reagents was, therefore, examined. The best results were encountered with chromium trioxide–sulfuric acid in acetone solution[9] which led to a neutral and an acidic product of which the former proved to be of great diagnostic value. While its analytical composition indicated that only oxidation of the secondary hydroxyl group to a carbonyl function had occurred, the optical rotatory dispersion curve[10] was plain and the absence of a Cotton effect made it very unlikely that the substance possessed a cyclic ketone grouping. The ultraviolet absorption spectum ($\lambda_{max}^{C_2H_5OH}$ 237 mμ) indicated the presence of the intact enol ether–carbomethoxy chromophore of genipin (I), but its infrared spectrum now exhibited a new, strong band at 5.64 μ. Its position is consistent with that of a γ-lactone grouping or a vinyl ester,[11] which would imply that partial structure A of genipin can be expanded to D, oxidation leading to a lactone (cyclic vinyl ester) E. Indeed, when this lactone E was exposed to alkali, it dissolved readily and upon acidification furnished a *dibasic* acid, lacking the methoxyl function of the starting material. Aside from saponification of the ester grouping, the elementary analysis indicated the loss of an additional carbon and oxygen atom, which can readily be rationalized by opening of the lactone ring of E to the intermediate F. Reverse Claisen condensation with loss of formate and saponification of the ester function are unexceptional and would lead from F to the dibasic acid G, completely consistent with the observed analytical results.

(9) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(10) C. Djerassi, *Optical Rotatory Dispersion: Applications to Organic Chemistry*, McGraw-Hill, New York, 1960.

(11) See L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Methuen, London, 1958, 2nd ed., Chap. 11.

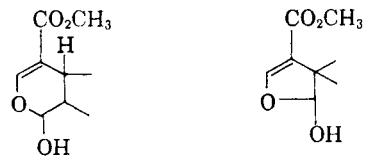


D E F G

In terms of the eventually deduced structure of genipin (I), the lactone possesses structure IV, while its alkali degradation product, the dibasic acid and its derived dimethyl ester can be assigned structures Va and Vb. The partial structure D in genipin (I) and in 10-deoxy-7,8-dihydrogenipin (IIa) also explains the course of the reaction of IIa with 2,4-dinitrophenylhydrazine, which affords a bis-2,4-dinitrophenylhydrazone (VI), whose ultraviolet spectroscopic properties[12] are only compatible with two isolated, non-conjugated 2,4-dinitrophenylhydrazone groupings of aliphatic aldehydes.

The acidic product from the chromium trioxide oxidation could not be purified readily and proved to be quite labile. Nevertheless, there seems to be little doubt that it can be assigned structure VIIa, since methylation afforded a dimethyl ester, whose analytical composition was consistent with formulation VIIb. Vacuum distillation of the acid VIIa led in part to the lactone IV, while treatment with alkali furnished the crystalline dibasic acid Va.

Partial structure D can be closed into the required[3] heterocyclic ring in two fashions, leading to the six-membered (H) or five-membered (J) alternatives. The former is more likely, because the NMR spectrum of genipin (*vide supra*) suggests the attachment of a CH function in the α position of the conjugated double bond, while the infrared spectral data of the oxidation product E could not be used[11] safely for purposes of differentiation.



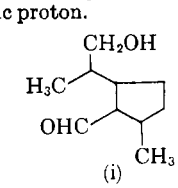
H J

In order to settle this point, it was intended to reduce the carbomethoxy function in 10-deoxy-7,8-dihydrogenipin (IIa) with lithium aluminum hydride and to remove the resulting primary hydroxyl group by hydrogenolysis to an intermediate suitable for further degradation. In actual fact, the lithium aluminum hydride reduction took a completely unexpected course, which will now be described. The principal product was an optically active, low-melting solid (m.p. 37–39°) of the composition $C_{10}H_{18}O_2$. Since the starting material IIa possesses the empirical formula $C_{11}H_{16}O_4$, the anticipated reduction of the carbomethoxy function was accompanied by additional loss of oxygen elsewhere in the molecule. The infrared spectrum exhibited no carbonyl absorption,[13] but two bands at 6.08 and 11.03μ, typical of a terminal double bond, were noted. The NMR spectrum confirmed the presence of a terminal methylene grouping by a pair of lines at δ = 5.20 and 4.99, while a peak at δ = 4.10 with intensity corresponding to two protons is assignable to the allylic protons on the carbon atom (C-11 of genipin) bearing the primary hydroxyl group. The two hydroxyl groups of this substance (subsequently shown to be VIIIa) appear to be exchanging and their resonance partially obscures some signals in the region near δ = 3.5. Consequently, the NMR spectrum (Fig. 3) of its diacetate VIIIb was studied and it will be noted that the peak attributable to the two allylic protons has shifted from δ = 4.10 to 4.56. This downfield shift of 0.46 p.p.m. is quite comparable to the change in δ of 0.36 p.p.m. (upon acetylation) for the two allylic protons attached to C_{10} of genipin (I). A doublet (spacing 6 c.p.s.) at δ = 3.88 is now observed, free of hydroxyl interference, which has shifted 0.38 p.p.m. downfield. Electronic integration of this doublet showed unequivocally that it corresponds to *two* protons. This information, together with the size of the downfield shift and the fact that the doublet lies 0.68 p.p.m. toward higher field than the allylic methylene group requires that the acetate VIIIb (and hence the parent alcohol (VIIIa) possesses two primary hydroxyl groupings, one allylic and the other nonallylic. Aside from the peaks at δ = 5.10 and 4.93 corresponding to the terminal methylene group and the doublet at δ = 1.00 attributable to the methyl group, the NMR spectrum (Fig. 3) also possesses sharp peaks at δ = 2.06 and 1.95 showing that the two acetate groups are nonequivalent. In the chronological sequence of events, the NMR analysis of the alcohol VIIIa and its diacetate VIIIb preceded the structure elucidation of the lactone IV described above and thus represented crucial evidence for the mode of attachment of the C-1 hydroxyl group in genipin (I).

Chemical support for the spectroscopic conclu-

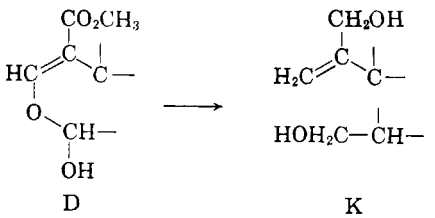
(12) E. A. Braude and E. R. H. Jones, *J. Chem. Soc.*, 498 (1945); J. D. Roberts and C. Green, *J. Am. Chem. Soc.*, **68**, 214 (1946); C. J. Timmons, *J. Chem. Soc.*, 2613 (1957).

(13) A minor product of the lithium aluminum hydride reduction was represented by a liquid, isomeric substance, which possessed a strong infrared carbonyl absorption, no exocyclic methylene group and two C-methyl functions. No further work was done but the most likely structure (i) is excluded by the NMR spectrum which showed the absence of an aldehydic proton.



(i)

sion of the presence of a terminal methylene function was adduced by ozonolysis of the diacetate VIIIb to a crystalline nor-ketone IX. The infrared absorption of the new keto group in IX was masked by the strong acetate band, but its presence was demonstrated by the analytical composition of the substance and most importantly, by the strong positive Cotton effect typical[10] of carbonyl groups. The NMR spectrum of the ketone IX proved to be very instructive, because it offered a means of distinguishing partial structure H from J. The conversion of the terminal methylene group to a carbonyl function results in a "magnetic" nonequivalence of the two protons in the nonallylic CH_2 group [corresponding to C-1 in genipin (I)]. A pattern of lines was observed over the range 224 to 262 c.p.s. from the internal tetramethylsilane reference, which was readily recognized as the AB part of an ABX spin-coupling pattern.[4a] The X proton is located on the ring (not possible in partial structure J) and its resonance is complex and is also obscured by the other ring protons. Thus observation of the ABX pattern in the NMR spectrum of IX removes all doubt from the assignment of two protons in the nonallylic alcoholic group of the precursor VIIIa and provides proof of the presence of a proton on the neighboring carbon atom (C-9). It is also pertinent to note that the allylic CH_2 group (now adjacent to carbonyl) shifted to $\delta = 4.78$, an additional shift of 0.22 p.p.m. arising from the greater unshielding ability of carbonyl compared to a carbon-carbon double bond. The principle course of the lithium aluminum hydride reduction therefore can be expressed in terms of the transformation D → K and at least two precedents for this type of enol ether cleavage have been recorded in the literature.[14]

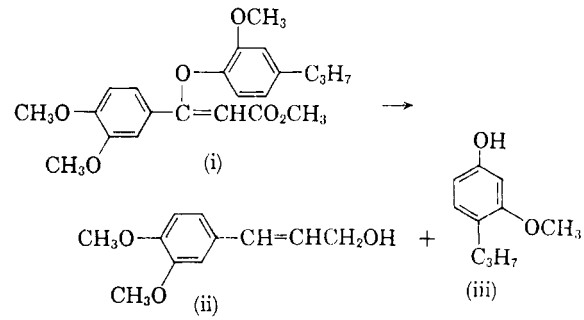


D K

According to partial structure K, which is required by the NMR spectral evidence, it should be possible to remove the primary allylic alcohol of VIIIa by hydrogenolysis. Indeed, when the alcohol VIIIa was exposed to hydrogenation in methanol solution in the presence of a palladized charcoal catalyst, two equivalents of hydrogen were consumed with formation of an alcohol (Xa), $C_{10}H_{20}O$, which possessed only a single hydroxyl function. Oxidation of this alcohol with chromium trioxide provided a liquid acid (Xb), $C_{10}H_{18}O_2$, which was further characterized as the methyl ester Xc and especially as the crystalline *S*-benzylthiouronium salt m.p. 121–122°, $[\alpha]_D$ −5.5°.

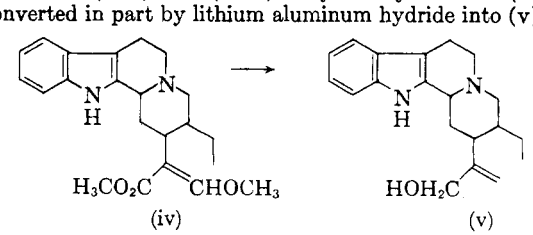
Turning now to partial structure H for 10-deoxy-7,8-dihydrogenipin (IIa) only four saturated carbon atoms remain, one of them a methyl group, which must be attached to H in order to construct the second ring known[3] to be present. The only manner in which this can be done is represented by partial structure L, with the methyl group occupying one methyl group occupying one of three positions in the cyclopentane ring. Successive exposure of a substance such as L to lithium aluminum hydride, catalytic hydrogenation, and finally oxidation, as mentioned in the preceding paragraph, then leads to structure M for the monobasic acid $C_{10}H_{18}O_2$. The methyl group in M can only be attached to one of the starred carbon atoms and of these three representations, only one (XII) follows the isoprene rule. In fact, structure XII (for absolute configuration see Ref. 15) corresponds to the hydrogenation-with-hydrogenolysis product[16] of nepetalactone (XI),[15,16] a naturally occurring monoterpene. The reduction of nepetalactone (XI) was, therefore, repeated according to the literature directions[16] and the resulting liquid acid XII transformed into its crystalline *S*-benzylthiouronium salt, m.p. 118–119°. The infrared spectrum of this salt was virtually identical with that of the corresponding salt, m.p. 121–122° derived from genipin, but their respective rotations differed somewhat (*S*-benzylthiouronium salt of Xb, $[\alpha]_D$ −5.5°; of XII,

(14) K. Freudenberg and G. Wilke, *Ber.*, **85**, 78 (1952) reported that in the lithium aluminum hydride reduction of (i), in addition to the anticipated primary alcohol, there were also isolated the two fission products (ii) and (iii).



(i) (ii) (iii)

According to P. Karrer, R. Schwyzer, and A. Flam, *Helv. Chim. Acta*, **35**, 851 (1952) dihydrocorynanthein (iv) is converted in part by lithium aluminum hydride into (v).

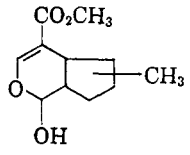


(iv) (v)

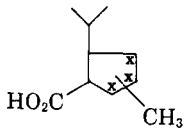
(15) R. B. Bates, E. J. Eisenbraun, and S. M. McElvain, *J. Am. Chem. Soc.*, **80**, 3420 (1958); E. J. Eisenbraun and S. M. McElvain, *J. Am. Chem. Soc.*, **77**, 3383 (1955); S. M. McElvain and E. J. Eisenbraun, *J. Am. Chem. Soc.*, **77**, 1599 (1955).

(16) J. Meinwald, *J. Am. Chem. Soc.*, **76**, 4571 (1954).

$[\alpha]_D$ +2.8°). The significance of this difference was confirmed by rotatory dispersion measurements,[10] the two salts exhibiting plain disperion curves of opposite sign.

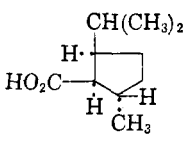


L

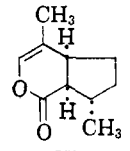


M

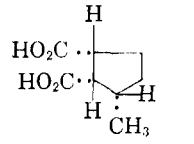
The above results strongly suggest that the acid Xb derived from genipin possesses the same structure as the acid XII obtained from nepetalactone, but that they differ in terms of their stereochemistry. Two approaches were open to settle this crucial point. The first would be to convert the carboxyl group of Xb into a ketone function, since the resulting cyclopentanone can be racemized[16] and compared with synthetic[17] 2-methyl-5-isopropylcyclopentanone. This route suffers from the disadvantage that it would not offer any information on the absolute configuration of the acid Xb and hence of genipin (I). The second approach involved possible correlation through the nepetic acids[15,18] where several stereoisomers are known, and it was selected for actual examination.



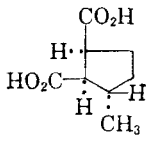
XII



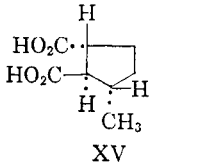
XI



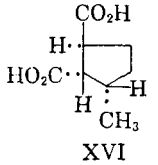
XVII



XIV



XV



XVI

For this purpose, 10-deoxy-7,8-dihydrogenipin (IIa) was ozonized in methylene chloride solution at −70° and the ozonide decomposed with alkaline hydrogen peroxide. The resulting crystalline acid was purified through its barium salt and then regenerated, whereupon it exhibited m.p. 136–137°, $[\alpha]_D$ −0.9° (chloroform[19]). In terms of elementary analysis, this acid (XIIIa) corresponded to the nepetic acids (XIV–XVII) but its physical constants and notably the negligible rotation in chloroform solution precluded identity with any of the three known[15] optically active nepetic acids ($[\alpha]_D$ in chloroform: XIV, +69.1°; XV, −35.4°; XVI, +85.8°). The remaining all *cis* isomer XVII is only reported[18] as the racemate, but it is pertinent to mention that Brewster,[20] on the basis of his rotation rules, has predicted a negligible rotation[19] for isomer XVII. This suggested that the dibasic acid from the ozonolysis of 10-deoxy-7,8-dihydrogenipin (IIa) may be the remaining unknown nepetic acid (XVII) or its antipode (XIIIa). Structurally, this conclusion was verified by infrared comparison of the dibasic acid XIIIa and its dimethyl ester XIIIb with the corresponding racemates of the *cis, cis*-nepetic acid.[18,21] Since the infrared spectra of all four racemic nepetic acids (racemates corresponding to XIV–XVII) have been found to show characteristic differences,[18] there remains no doubt that the dibasic acid from the genepin series must be either XIIIa or its antipode XVII.

In order to settle this remaining question, the dibasic acid XIIIa was converted into its anhydride, treated with methanol to afford the half ester, and then heated under reflux with methanolic sodium methoxide in order to epimerize the carbomethoxy function. The crude, epimerized half-ester (XVIIIb) was then saponified to give a new nepetic acid, which should correspond either to the known nepetic acid XVI or its antipode XVIIIa. Bates, Eisenbraun, and McElvain[18] have shown that such a sequence can be employed to convert the racemate of the *cis,cis*-nepetic acid (XVII) into racemic *trans,cis*-nepetic acid (XVI). The isomerized acid exhibited m.p. 95–100°, $[\alpha]_D$ −66.2° as compared to m.p. 114–115°, $[\alpha]_D$ +85.8° for XVI.[15] We can conclude,[22] therefore, that the isomerized dibasic acid in the genipin series should be represented by stereoformula XVIIIa, its precursor, the

(17) J. Golé, *Bull. Soc. Chim. France*, 894 (1949).

(18) R. B. Bates, E. J. Eisenbraun, and S. M. McElvain, *J. Am. Chem. Soc.*, 80, 3413 (1958).

(19) A considerable solvent dependence was noted, the rotation being considerably higher (+37°) in methanol and this weakens somewhat the validity of applying rotation rules (Ref. 20) to acids of this series.

(20) J. H. Brewster, *J. Am. Chem. Soc.*, 81, 5483 (1959).

(21) We are indebted to Prof. S. M. McElvain and Dr. C. B. Abrahams of the University of Wisconsin for this valuable gift.

(22) The fact that the isomerized acid could not be obtained with a narrower melting point range and that the magnitude of its rotation did not correspond exactly to that of the antipodal acid XVI is probably due to the fact that methanolysis of the anhydride did not give exclusively one monomethyl ester. The final product would, therefore, be contaminated by some of the antipode of the *trans, trans*-nepetic acid (XV), thus resulting in a less negative rotation of XVIIIa. It should be noted that the optically active nepetic acid (XVI), whose constants are used for comparison with the antipode XVIIIa, was not prepared by such an isomerization sequence but rather by oxidation of the corresponding nepetonic acid (see Ref. 15). In any event, the change in rotation accompanying the epimerization of the *cis,cis*-acid XIIIa is of such an order of magnitude as to preclude any structure other than XVIIIa as the principal product.

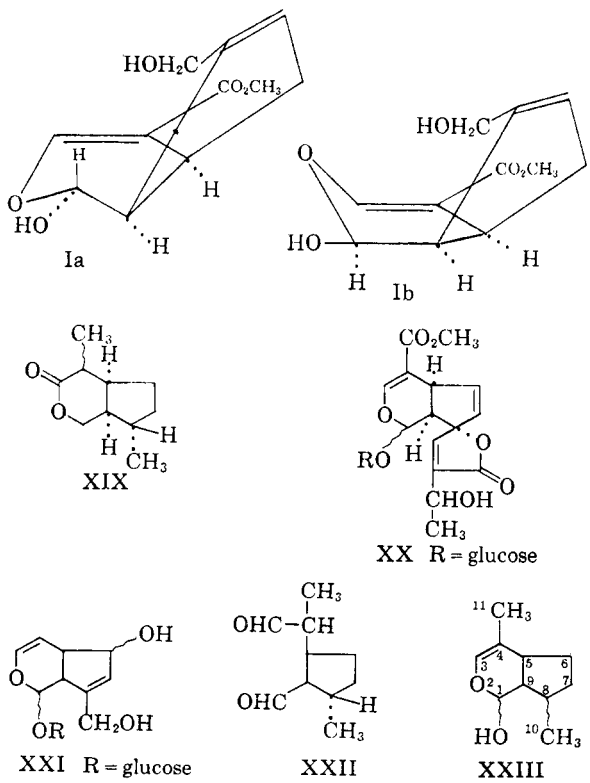
cis,cis-dibasic acid by XIIIa, and hence 10-deoxy-7,8-dihydrogenipin by IIa.

Complete structure of genipin (I) *and biogenetic considerations.* With the establishment of the structure and absolute configuration of 10-deoxy-7,8-dihydrogenipin, the constitution of genipin follows automatically from the information already available. The conversion of genipin into 10-deoxy-7,8-dihydrogenipin (IIa) involves the removal of a primary hydroxyl group with the generation of a methyl substituent as well as reduction of a double bond allylic to that hydroxyl function. Since 10-deoxy-7,8-dihydrogenipin (IIa) possesses only one C-methyl group, the primary hydroxyl function must be attached to it (C-10 in structure I). This leaves only two locations for the double bond, which suffered saturation in the conversion of genipin to IIa, namely between carbon atoms 7 and 8 or between positions 8 and 9. A differentiation can be made readily on the basis of the NMR spectrum (Fig. 1) of genipin, since this shows clearly the presence of one proton on that double bond and this is only possible in the 7-8 location of the double bond. This completes the assignment of the structure and absolute configuration of genipin in terms of the expression I,[23] with the exception of the secondary hydroxyl group attached to C-1. The NMR spectrum (Fig. 1) demonstrated the equatorial orientation of this hydroxyl group, but it can nevertheless be assigned to α- or β-notation,[23] depending upon whether genipin possesses conformation IA or IB.

Biogenetically, genipin belongs to a very interesting group of monoterpenoids. This includes nepetalactone (XI),[15,16] the ant lactones[24] (e.g. iridomyrmecin (XIX)), plumieride (XX),[6] and aucubin (XXI).[25] In all cases where the absolute configuration of the ring juncture has been established (I, XI, XIX, XX) this has proved to be identical and it is very likely that this will also apply to aucubin (XXI).

It has been suggested[26] that the naturally occurring [27] iridodial (XXII) is the biological precursor of iridomyrmecin (XIX) involving disproportionation of the dialdehyde. Such dialdehydes may also represent the precursors of the other monoterpenoids of this series, direct cyclization rather than disproportionation leading to the enol–half acetal XXIII. Oxidation at C-1 would then furnish nepetalactone (XI), while allylic oxidation at C-1 would result in production of the carboxyl group of genipin (I) and plumieride (XX). In aucubin, C-11 has been lost, most likely by decarboxylation. Hydroxylation at C-6 and/or C-10 (I, XX, XXI) as well as introduction of unsaturation in the cyclopentane ring are probably secondary processes, which may happen either before or after generation of the dihydropyran ring. This probably also applies to the attachment of the additional four carbon atoms of plumieride (XX) involved in the third ring; Halpern and Schmid[6] have already noted that these arise probably from acetoacetate and a precursor, which can now be seen to have a very close structural resemblance to genipin (I).

Structure of some hydrogenation products. In the introductory section, it was pointed out that the catalytic hydrogenation of genipin (I) yielded a mixture, from which five pure constituents could be separated. While the structure of one of them, 10-deoxy,7,8-dihydrogenipin (IIa), sufficed for the establishment of the constitution of genipin (I),



(23) Throughout this paper we are using absolute configurational representations following the steroid notation.

(24) For leading references see R. Fusco, R. Trave, and A. Vercellone, *Chim. e Ind.* (*Milano*), **37**, 251, 958 (1955); G. W. K. Cavill and H. D. Locksley, *Austral. J. Chem.*, **10**, 352 (1957); R. H. Jaeger and R. Robinson, *Tetrahedron Letters*, No. 15, 14 (1959); T. Sakan, A. Fujino, F. Murai, A. Suzui, and Y. Butsugan, *Bull. Chem. Soc. Japan*, **32**, 1154 (1959).

(25) S. Fujise, H. Obara, and H. Uda, *Chem. & Ind.* (*London*), 289 (1960); see also P. Karrer and H. Schmid, *Helv. Chim. Acta*, **29**, 525 (1946); J. Grimshaw and H. R. Juneja, *Chemistry & Ind.* (*London*), 656 (1960). M. W. Wendt, W. Haegele, E. Simonitsch and H. Schmid, *Helv. Chim. Acta*, **43**, 1440 (1960) have pointed out that in addition to structure XXI for aucubin an alternate expression is possible in which the C-6 hydroxyl group is placed at C-7 and the double bond is located between C-8 and C-9.

(26) K. J. Clark, G. I. Fray, R. H. Jaeger, and R. Robinson, *Tetrahedron*, **6**, 217 (1959).

(27) G. W. K. Cavill, D. L. Ford, and H. D. Locksley, *Chem. & Ind.* (*London*), 465 (1956).

parallel work was also conducted with the other products as discussed below.

Hydrogenation of genipin (I) in methanol solution in the presence of palladized charcoal catalyst led to three substances which could be separated by a combination of distillation and chromatography. The most polar product was 10-deoxy-7,8-dihydrogenipin (IIa), which has already been covered in the preceding section. The least polar one, now named 1,10-bisdeoxy-7,8-dihydrogenipin (XXIVa) initially presented considerable difficulties, since no satisfactory analytical results could be obtained. Apparently this is due to the hygroscopic nature of the substance, whose structure XXIVa was demonstrated as follows. The presence of the intact chromophore involving the carbomethoxy moiety and the enol ether grouping was confirmed by the characteristic infrared and ultraviolet spectroscopic properties. Saponification of XXIVa led to the free acid XXIVb, which formed a crystalline *S*-benzylthiouronium salt and which upon regeneration provided a specimen of the oily acid XXIVb with analytical figures consistent with the empirical formula $C_{10}H_{14}O_3$. Remethylation with diazomethane again provided 1,10-bisdeoxy-7,8-dihydrogenipin (XXIVa). Heating of the free acid resulted in decarboxylation, while treatment with 2,4-dinitrophenylhydrazine yielded a bis-2,4-dinitrophenylhydrazone corresponding in terms of analytical composition and ultraviolet spectroscopic properties with structure XXV. Apparently, opening of the dihydropyran ring was accompanied by oxidation of the intermediate hydroxyaldehyde to the dialdehyde.

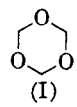
Further hydrogenation of XXIVa could be effected with platinum oxide in acetic acid solution which resulted in the uptake of one equivalent of hydrogen and the saturation of the double bond. The oily product, 1,10-bisdeoxy-3,4,7,8-tetrahydrogenipin (IIIa), upon saponification afforded the free acid IIIb, which formed a crystalline *S*-benzylthiouronium salt. The same salt had already been obtained earlier[3] from genipin (I) without isolation of intermediates by successive hydrogenation with palladium-charcoal and then platinum oxide in acetic acid. These reactions, notably the conversion to 1,10-bisdeoxy-3,4,7,8-tetrahydrogenipin (IIIa), settle the constitution of 1,10-bisdeoxy-7,8-dihydrogenipin (XXIVa).

A third substance, of intermediate polarity between XXIVa and IIa, could be separated in crystalline form from the palladium–methanol hydrogenation of genipin. Its empirical formula corresponded to $C_{13}H_{22}O_5$ and no free hydroxyl or enol ether absorption was noted in the infrared. Treatment with hydroxylamine or semicarbazide under alkaline conditions did not afford any carbonyl derivative, but exposure to 2,4-dinitrophenylhydrazine in acid solution yielded the bis-2,4-dinitrophenylhydrazone VI, which had already been obtained by a similar reaction from 10-deoxy-7,8-dihydrogenipin (IIa). The isolation of the bis-2,4-dinitrophenylhydrazone VI demonstrates the unsubstituted nature of the cyclopentane ring and requires that the additional two carbon atoms must have entered into the pyran ring. The NMR spectrum (Fig. 4) immediately settled the structure of the product as 3-methoxy-10-deoxy-3,4,7,8-tetrahydrogenipin 1-methyl ether (XXVI) since its three peaks at δ = 3.50, 3.54, and 3.70 can all be assigned to methoxy group resonances and this was confirmed by Zeisel methoxyl analysis. The formation in an acid medium of the bis-2,4-dinitrophenylhydrazone VI from such a structure is, of course, unexceptional. The NMR peak at δ = 3.70, which is distinctly different from the other two, lies close to the value (3.73) observed for the carbomethoxy group in genipin (Fig. 1). A pair of lines is found at 259 and 267 c.p.s. (δ = 4.39) and a similar pair at 276 and 284 c.p.s. (δ = 4.66) with intensities corresponding to one proton for each pair. The size of the spin coupling, 8 c.p.s., shows that each proton is axial and possesses one axial neighbor in a six-membered saturated ring. The equality of intensities of the lines in each pair indicates that the two protons in question are not coupled to one another and hence are not on adjacent carbon atoms. Furthermore, the doublets are found in a region of the spectrum which is characteristic of CH groups adjacent to two oxygen atoms.

Catalytic hydrogenation of genipin (I) in glacial acetic acid in the presence of platinum oxide catalyst furnished three crystalline substances. The most abundant one (32% yield) was 10-deoxy-7,8-dihydrogenipin (IIa), a second one was isolated in 8% yield, while the third one was only encountered in trace quantities.

This second product from the platinum oxide–acetic acid hydrogenation was less polar than 10-deoxy-7,8-dihydrogenipin (IIa). Its empirical formula ($C_{11}H_{14}O_4$) differed by only two hydrogen atoms from that of IIa ($C_{11}H_{16}O_4$) and the new product was characterized by its unusually high positive rotation ($[\alpha]_D$ +161°). Its infrared spectrum still exhibited the bands at 5.89 and 6.09 μ associated with the carbomethoxy and enol ether functions, respectively, but it did not show any absorption due to a hydroxyl group. The fourth oxygen must, therefore, be ethereal in nature. The ultraviolet absorption maximum at 234 mμ was somewhat displaced to shorter wave length, as compared to genipin (I). The simplest structure consistent with the above data is the tricyclic one, 1,10 - anhydro - 7,8 - dihydrogenipin (XXVII), in which a new oxide ring had been produced between C-1 and C-10, since the substance did not possess a C-methyl function (in contrast to the other hydrogenation products IIa, XXIVa, and

XXVI) by Kuhn-Roth oxidation. Stereochemically, such ring closure appears perfectly feasible by inspection of models and the NMR spectrum (Fig. 5) confirms such a formulation. The characteristic peak of the proton attached to C-3 (δ = 7.53 and I and 7.41 in IIa) can be found in the expected region at δ = 7.53. The doublet at δ = 5.33 with a spacing of 5 c.p.s. can be assigned to the proton at C-1, since it is adjacent to two oxygen atoms.[28]



(I)

The carbomethoxy peak is found at δ = 3.73 falling among a group of lines which can be recognized as the AB part of an ABX multiplet, thus confirming the presence of a CH_2 group (C-10) in a ring adjacent to oxygen and possessing only one adjacent proton neighbor (attached to C-8).

In view of the unusual behavior of 10-deoxy-7,8-dihydrogenipin (IIa) towards lithium aluminum hydride, it appeared of interest to examine the corresponding cyclic ether XXVII under identical conditions. The minor product of this reaction proved to be the unsaturated triol XXIXa, formally generated by cleavage of the enol ether grouping and complete reduction of the carbomethoxy and masked aldehyde functions. The triol XXIXa was not analyzed as such, but transformed into its triacetate XXIXb, which gave satisfactory analytical figures. The infrared spectrum showed the presence of acetate groups as well as the terminal olefin, while its NMR spectrum was quite unambiguous, the triacetate XXIXb simply representing the analog of the diacetate VIIb with an additional acetoxy group attached to the methyl substituent. Consequently, the only significant differences in the NMR spectra of the diacetate VIIIb (Fig. 3) and the triacetate XXIXb are the appearance of a third (methyl) acetate group at δ = 2.05 and the disappearance of the methyl doublet of VIIIb, which is now replaced by a complex CH_2 multiplet centered at about δ = 3.84.

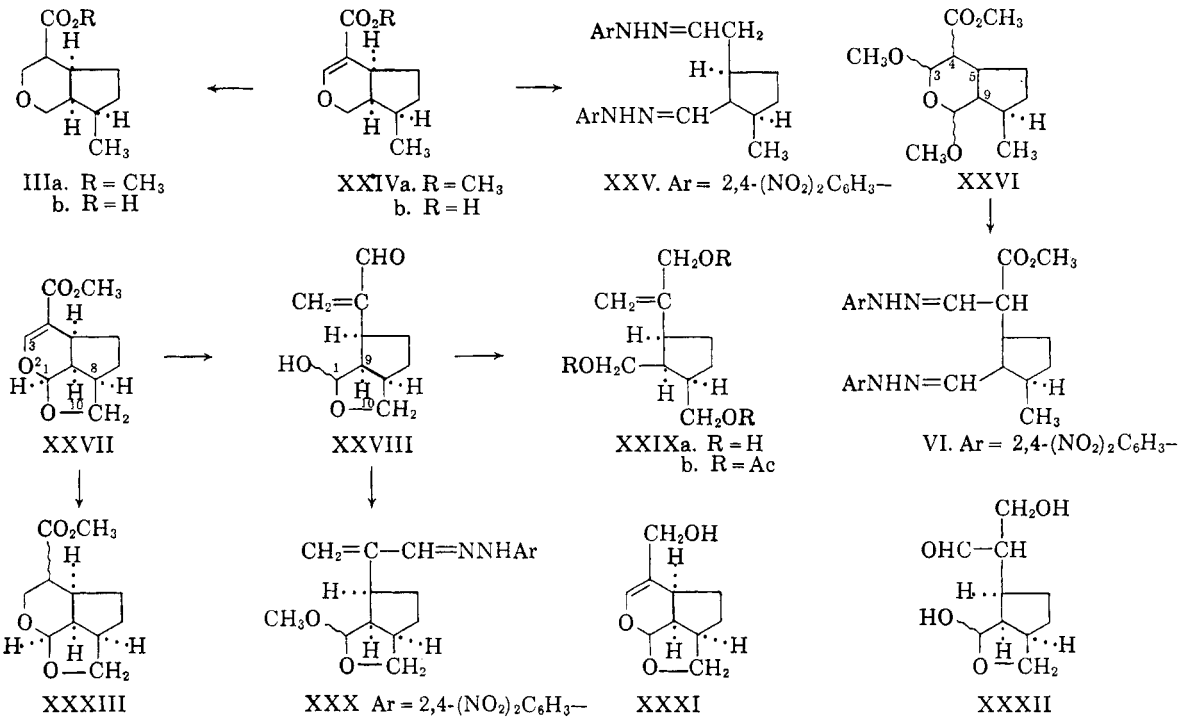
The principal product in the lithium aluminum hydride reduction turned out to be the α,β-unsaturated aldehyde XXVIII. The analytical composition of the oil was consistent with this formulation and the presence of the α,β-unsaturated aldehyde was indicated by the ultraviolet and infrared spectral properties of the substance. The existence of the cyclic hemiacetal grouping was demonstrated by treatment with 2,4-dinitrophenylhydrazine in *methanolic* hydrochloric acid, whereupon the 2,4-dinitrophenylhydrazone XXX of the methyl ether of XXVIII was obtained, the introduction of a methoxyl group being established by Zeisel determination. The interpretation of the NMR spectrum, though somewhat complicated, lends support to structure XXVIII. An aldehyde peak is observed at δ = 9.58 and a pair of lines at δ = 6.11 and 6.30 represent the protons on the terminal methylene group.[29] A rather poorly resolved multiplet is found, centered at about δ = 3.83, which seems to be characteristic of the two protons of the methylene group (C-10) in the five-membered ring adjacent to oxygen. The peak at δ = 4.86 was first suspected of arising from the proton on the hydroxyl group, but its temperature and concentration independence require that it be assigned instead to the hydrogen atom attached to C-1. The proton on this same carbon atom in XXVII (Fig. 5) is found at δ = 5.33 and the reduced shielding in XXVII relative to its reduction product XXVIII or trioxan[28] may be due to the six-membered unsaturated ring, which may result in some long range effects. A small sample of XXVIII was acetylated and the NMR spectrum of the total crude product examined. The major change in the spectrum was a shift of this peak (due to proton at C-1) from δ = 4.86 to 5.71, thus confirming its assignment.[30]

It would be attractive to assume that the α,β-unsaturated aldehyde XXVIII is an intermediate in the formation of the triol XXIXa by lithium aluminum hydride reduction of 1,10-anhydro-7,8-dihydrogenipin (XXVII). In fact, further reduction of the aldehyde XXVIII led directly to the triol XXIXa. Nevertheless, no reasonable mechanism for the sequential production of XXVIII and XXIX seems available. The simplest rationalization would be assumption of initial reduction of the carbomethoxy group to XXXI followed by cleavage of the acetal grouping to XXXII when the reaction mixture is decomposed. Dehydration of the β-hydroxyaldehyde in XXXII would then lead to XXVIII. Two difficulties immediately arise if one visualizes such a picture. First, the decomposition was conducted with aqueous sodium sulfate and no acid was employed, thus implying that the acetal linkage in XXXI is unusually labile. Secondly,

(28) Trioxan (i) exhibits a peak at δ = 5.00 (observation by G. V. D. Tiers).

(29) The fact that they are 1.2 p.p.m. less magnetically shielded as compared to VIIIb (Fig. 3) indicates that the terminal methylene group is conjugated, presumably with the aldehyde.

(30) The lack of doubling of the resonance of the proton attached to C1 through spin-spin coupling to the adjacent proton at C9 can be rationalized as follows. Recent theoretical work (M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959)) has shown that there is quite a range (70–100°) for the dihedral angle between the H—C—C planes for two protons on adjacent carbon atoms which leads to the expectation of a near-zero spin coupling. In fact, the coupling for the proton attached to C-1 in XXVII (Fig. 5) is slightly less than 5 c.p.s. which would correspond to a dihedral angle near 120°. Reduction of the angle from 120° to 100° upon opening of the six-membered ring (XXVII → XXVIII) would reduce the spin coupling to an unresolvable value. Therefore, the absence of observable doubling of the resonance in question need not be regarded as an obstacle to the explanation of the NMR spectrum in terms of structure XXVIII.



such a mechanism does not explain the formation of the triol XXIXa since generation of the intermediate XXXII *after* decomposition of the lithium aluminum hydride does not permit further reduction of the aldehyde and hemicacetal groupings. We believe, therefore, that the unsaturated aldehyde XXVIII is not an intermediate in the formation of the triol XXIXa. The latter apparently arises from cleavage of the 2–3 bond[14] followed by further reduction of the carbomethoxy and hemiacetal functions. As far as the production of the aldehyde XXVIII is concerned, we are reduced to assume that an intermediate of type XXXI is unusually labile to hydrolytic opening yielding XXXII.

In some platinum oxide–acetic acid hydrogenations of genipin (I), in addition to IIa and XXVII there was also encountered in trace amounts a third crystalline product. Its empirical formula ($C_{11}H_{16}O_4$) differed by only two hydrogen atoms from that of the cyclic acetal XXVII and like it, the substance possessed a high positive rotation and showed no hydroxyl absorption in the infrared. The most significant difference resided in the position of the carbomethoxy peak, which now occurred at 5.79 μ and the absence of any selective ultraviolet absorption maximum. The most likely structure satisfying all these requirements is that expressed by 1,10-anhydro-3,4,7,8-tetrahydrogenipin (XXXIII) and this was confirmed by hydrogenation of the unsaturated precursor XXVII with platinum oxide in acetic acid.

EXPERIMENTAL[31]

Nuclear magnetic resonance measurements. All compounds were studied as dilute solutions in deuterochloroform unless otherwise specified. A trace of tetramethylsilane was added to the solvent to serve as an internal reference. Peak positions were measured in c.p.s. relative to the reference by the usual audio side-band technique, using a Hewlett-Packard 200 CD audio oscillator. Frequencies were checked with an HP 521C electronic counter.

The NMR instrument was a Varian Associates HR-60 High Resolution Spectrometer. Thin-walled sample cells (0.195″ o.d., 0.165″ i.d.) were employed for maximum signal-to-noise with dilute solutions. The magnetic field strength was 14,092 oersteds, corresponding to a proton resonance frequency of 60 mc. Spectra were swept from low to high field. We are indebted to Mr. LeRoy Johnson for all of the NMR measurements.

Hydrogenation of genipin (I) *with platinum oxide in acetic acid solution.* A solution of 30 g. of genipin (I)[3] in 250 cc. of glacial acetic acid was hydrogenated at room temperature and atmospheric pressure in the presence of 6.0 g. of prereduced platinum oxide catalyst. Hydrogen uptake corresponding to 2 equivalents occurred within 9 hr., whereupon the catalyst was filtered and most of the acetic acid was removed *in vacuo*. The residue was taken up in ether, washed with aqueous sodium carbonate solution, water, dried, and evaporated to yield 27 g. of a slight yellowish oil. Crystallization from ether–petroleum ether afforded 6.55 g. of *10-deoxy-7,8-dihydrogenipin* (IIa), m.p. 81–82.5°, which together with an additional 2.60 g. obtained from chromatography of the mother liquors raised the yield to 32%. The analytical sample crystallized from the same solvent pair as leafy plates, m.p. 81.5–83°, $[\alpha]_D$ −2.4° (*c*, 1.22 in methanol), $\lambda_{max}^{C_2H_5OH}$ 240 mμ, log ϵ 4.06, $\lambda_{max}^{CHCl_3}$ 2.76, 2.93, 5.90, 6.11, and 11.09 μ, plain negative rotatory dispersion curve[10] ($[\alpha]_{500}$ −50°, $[\alpha]_{400}$ −126°, $[\alpha]_{300}$ −475°, $[\alpha]_{285}$ −690°) in methanol solution (*c*, 0.12).

(31) All melting points were determined on the Kofler block. We are indebted to Miss B. Bach for most of the ultraviolet and infrared spectral measurements, to Mrs. A. James and Mrs. T. Nakano for the optical rotatory dispersion curves, to Dr. A. Bernhardt (Mülheim, Germany) for the microanalyses and to Dr. D. K. Cox (Syntex, S.A., Mexico City) for collecting the plant material.

Anal. Calcd. for $C_{11}H_{16}O_4$: C, 62.25; H, 7.60; O, 30.15; OCH_3, 14.61; C—CH_3, 7.15. Found: C, 62.16; H, 7.37; O, 30.45; OCH_3, 14.31; C—CH_3, 6.45.

A sample was acetylated with pyridine and acetic anhydride at room temperature (16 hr.) and since *10-deoxy-7,8-dihydrogenipin acetate* (IIb) could not be crystallized, it was distilled for analysis; $\lambda_{max}^{CHCl_3}$ 5.72, 5.87, 6.11, and 8.10 μ.

Anal. Calcd. for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14; O, 31.46; C—CH_3, 11.78. Found: C, 61.64; H, 7.30; O, 31.86; C—CH_3, 11.76.

A 54-mg. portion of 10-deoxy-7,8-dihydrogenipin was treated with 2.0 cc. of 2,4-dinitrophenylhydrazine solution (1.0 g. of 2,4-dinitrophenylhydrazine, 7.5 cc. of concd. sulfuric acid, 75 cc. of ethanol diluted with water to a volume of 250 cc.) and the resulting precipitate chromatographed on 2.0 g. of acid-washed alumina. Elution with benzene and recrystallization from the same solvent afforded leafy orange plates, m.p. 176.5–177.5°, $[\alpha]_D$ +68° (*c*, 0.58 in chloroform), $\lambda_{max}^{CHCl_3}$ 348 mμ, log ϵ 4.63, which corresponded to the *bis-2,4-dinitrophenylhydrazone* VI of 1-methylcyclopentane-2-carboxaldehyde-3-(α-methoxycarbonyl) acetaldehyde.

Anal. Calcd. for $C_{23}H_{24}N_8O_{10}$: C, 48.25; H, 4.22; N, 19.57; O, 27.95. Found: C, 48.20; H, 4.22; N, 19.58; O, 27.91.

The mother liquor from the crystallization of 10-deoxy-7,8-dihydrogenipin (IIa) was chromatographed on 270 g. of Merck acid-washed alumina. The first benzene-ether (9:1) eluates afforded after crystallization from petroleum ether (b.p. 30–60°) at −70° approximately 40 mg. of *1,10-anhydro-3,4,7,8-tetrahydrogenipin* (XXXIII), which exhibited m.p. 42–42.5°, $[\alpha]_D$ +113° (methanol), $\lambda_{max}^{CHCl_3}$ 5.79 μ, no selective ultraviolet absorption.

Anal. Calcd. for $C_{11}H_{16}O_4$: C. 62.25; H, 7.60; OCH_3, 14.61. Found: C, 62.28; H, 7.55; OCH_3, 14.72.

The other benzene-ether (9:1 and 3:1) fractions were combined and crystallized from ether–petroleum ether leading to 2.24 g. (8%) of *1,10-anhydro-7,8-dihydrogenipin* (XXVII), m.p. 60–61.5°, $[\alpha]_D$ +161° (*c*, 1.12 in methanol), $\lambda_{max}^{C_2H_5OH}$ 234 mμ, log ϵ 4.10, $\lambda_{max}^{CHCl_3}$ 5.89, and 6.09 μ.

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H. 6.71; O, 30.44; OCH_3, 14.76. Found: C, 62.97; H, 6.79; O, 30.11; OCH_3, 14.80; C—CH_3, 0.0.

Final elution with ether and 9:1 ether-methanol provided an additional 2.60 g. of 10-deoxy-7,8-dihydrogenipin (IIa) of m.p. 81–82.5°.

Hydrogenation of 1,10-anhydro-7,8-dihydrogenipin (XXVII). 1,10-Anhydro-7,8-dihydrogenipin (XXVII) (0.367 g.) was hydrogenated in 40 cc. of glacial acetic acid with 70 mg. of prereduced platinum oxide at atmospheric pressure and 30°. Hydrogen uptake did not cease until 6 hr., whereupon 1.9 molar equivalents had been consumed indicating reduction beyond simple saturation of the double bond. The catalyst was filtered, the filtrate was diluted with a large amount of water and extracted with ether. The ether solution was washed with water, 5% aqueous sodium bicarbonate, then water, dried, and evaporated. The resulting colorless oil (0.23 g.) was crystallized from ether–petroleum ether (while cooling in Dry Ice) to afford 68 mg. of 1,10-anhydro-3,4,7,8-tetrahydrogenipin (XXXIII), m.p. 41–43°. Its infrared spectrum (chloroform) was identical with that of the sample isolated in the direct hydrogenation of genipin.

Hydrogenation of genipin (I) *with palladium-charcoal in methanol solution.* A solution of 8.32 g. of genipin (I) in 170 cc. of methanol was hydrogenated at room temperature and atmospheric pressure in the presence of 10% palladized charcoal catalyst, approximately 2 molar equivalents of hydrogen being consumed over a period of 20 hr. The "non-polar" constituents were separated by chromatography on basic alumina (Alcoa, grade F-20) and elution with benzene, thus affording 2.14 g. of oil. This material was fractionated at 1.0 mm. pressure into two major portions, (a) b.p. 91–97°/1.0 mm. and (b) b.p. 113–116°/1.0 mm.

Fraction (a) was redistilled twice and material boiling constantly at 85°/0.4 mm., corresponded to *1,10-bisdeoxy-7,8-dihydrogenipin* (XXIVa), n_D^{24} 1.4853, $\lambda_{max}^{CHCl_3}$ 5.87 and 6.10 μ, $\lambda_{max}^{C_2H_5OH}$ 238 mμ, log ϵ 3.91, $[\alpha]_D$ +46.7° (methanol), plain positive rotatory dispersion curve ($[\alpha]_{400}$ +71°, $[\alpha]_{300}$ +150°, $[\alpha]_{285}$ +230°) in methanol (*c*, 0.13).

Anal. Calcd. for $C_{11}H_{16}O_3 \cdot 1/3\ H_2O$: C, 65.32; H, 8.30; O, 26.35; C—CH_3, 7.43. Found: sample (1): C, 65.86; H, 8.28; O, 26.36; sample (2): C, 66.15; H, 8.35; O, 25.56; C—CH_3, 6.03.

The poor analytical results appeared to be due to the hygroscopic nature of the oil XXIVa as demonstrated as follows. Saponification of a portion of the oily methyl ester XXIVa by heating under reflux for 3 hr. with 0.5*N* ethanolic potassium hydroxide solution afforded in 92% yield an oily acid (XXIVb), which was converted into its *S*-benzylthiouronium salt by dissolving in 10% aqueous sodium hydroxide, adjusting the *p*H to 6.5 and adding the filtered solution to a saturated aqueous solution of *S*-benzylthiourea hydrochloride. Recrystallization from hot water provided an analytical specimen of the salt, m.p. 143–144°.

Anal. Calcd. for $C_{18}H_{24}N_2O_3S$: C, 62.05; H, 6.94; N, 8.04; O, 13.77; S, 9.20. Found: C, 61.62; H, 7.29; N, 7.91; O, 14.00; S, 8.99.

The above *S*-benzylthiouronium salt (94 mg.) was dissolved in 50% aqueous methanol and passed through a column of Dowex 50 ion exchange resin. The eluate was evaporated on the water pump to remove methanol and the aqueous solution was extracted with ether. Washing with sodium bicarbonate solution, acidification and re-extraction with ether provided 46 mg. of the *acid XXIVb*, which was distilled at 0.005 mm. for analysis; $\lambda_{max}^{CHCl_3}$ 5.94, 6.14 μ and typical "acid" absorption in 3–4 μ region.

Anal. Calcd. for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74; O, 26.34. Found: C, 65.74; H, 7.83; O, 26.66.

The acid XXIVb (22 mg.)[32] was now methylated with diazomethane and the resulting ester (pure XXIVa) redistilled; $\lambda_{max}^{CHCl_3}$ 5.87 and 6.12 μ. The analytical results were essentially identical to those of 1,10-bisdeoxy-7,8-dihydrogenipin (XXIVa) isolated directly from the hydrogenation.

Anal. Found: C, 65.45; H, 8.16; O, 26.21.

Treatment of an ethanolic solution of the acid XXIVb with 2,4-dinitrophenylhydrazine in sulfuric acid–ethanol–water afforded after chromatographic purification and recrystallization from benzene orange needles of the *bis-2,4-dinitrophenylhydrazone* XXV of 1-methylcyclopentane-2,3-dicarboxaldehyde, m.p. 219.5–220.5°, $[\alpha]_D$ −21.8° (*c*, 0.22 in chloroform), $\lambda_{max}^{CHCl_3}$ 352 mμ, log ϵ 4.74.

Anal. Calcd. for $C_{21}H_{22}N_8O_8$: C, 49.03; H, 4.31; N, 21.78; O, 24.99. Found: C, 49.29; H, 4.24; N, 21.63; O, 24.62.

The higher boiling fraction (b) from the distillation of the original "non-polar" hydrogenation products after two redistillations afforded *3-methoxy-10-deoxy-3,4,7,8-tetrahydrogenipin 1-methyl ether* (XXVI), b.p. 111–112.5°/1.2 mm., n_D^{25} 1.4682. The same substance was also obtained (eventually in crystalline form) by washing the original basic alumina chromatogram column with ether-methanol mixtures and re-chromatographing the "polar" fraction on Brockmann neutral alumina (activity VI). The hexane-benzene eluates gave the dimethoxy compound XXVI, while development of the column with ether-benzene (1:1), ether and chloroform produced *10-deoxy-7,8-dihydrogenipin* (IIa). Further purification of the latter was best effected by chromatography on silica gel and elution with chloroform. The resulting product was identical in all respects with the specimen obtained by the preferred platinum oxide–acetic acid procedure.

Crystallization of *3-methoxy-10-deoxy-3,4,7,8-tetrahydro-*

(32) Heating of the acid above 145° causes partial decarboxylation as was shown in a larger scale experiment: heating of 388 mg. of XXIVb at 145–150° and sweeping with dry nitrogen (free of oxygen and carbon dioxide) liberated 50 mg. of carbon dioxide, which was weighed by absorption on ascarite.

genipin 1-methyl ether (XXVI) could be effected from hexane to afford an analytical sample, m.p. 61–62°, $\lambda_{max}^{CHCl_3}$ 5.79 μ, which lacked selective ultraviolet absorption.

Anal. Calcd. for $C_{13}H_{22}O_5$: C, 60.44; H, 8.59; O, 30.97; OCH_3, 36.05; C—CH_3, 5.82. Found: C, 60.78; H, 8.49; O, 30.66; OCH_3, 39.25; C—CH_3, 4.64.

Treatment of a sample of XXVI with 2,4-dinitrophenylhydrazine as reported above for 10-deoxy-7,8-dihydrogenipin (IIa) produced the same bis-2,4-dinitrophenylhydrazone VI, m.p. 176–178°, undepressed upon admixture with a sample prepared from IIa. The infrared spectra of the two 2,4-dinitrophenylhydrazones were undistinguishable.

1,10-Bisdeoxy-3,4,7,8-tetrahydrogenipin (IIIa). (a) *By Raney nickel hydrogenation of 10-deoxy-7,8-dihydrogenipin* (IIa). A mixture of 218 mg. of 10-deoxy-7,8-dihydrogenipin (IIa) and 0.6 g. of W-2 Raney nickel catalyst (7 months old) in 25 cc. of ethanol was shaken for 24 hr. in the presence of hydrogen at 1500 p.s.i. and 200°. The resulting colorless liquid did not exhibit any more an infrared band near 6.10 μ corresponding to the enol ether and after chromatography on 7.5 g. of neutral Brockmann alumina (activity VI) there was eluted with benzene-chloroform (1:1) a liquid, which was distilled at 0.1 mm. for analysis; $\lambda_{max}^{CHCl_3}$ 5.79 μ with a very small band at 2.82 μ, indicating the presence of a small amount of hydroxyl-containing impurity, which is also indicated by the analytical results. The substance did not exhibit any selective ultraviolet absorption.

Anal. Calcd. for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15; O, 24.21. Found: C, 65.97; H, 9.04; O, 25.06.

(b) *By platinum oxide-acetic acid hydrogenation of 1,10-bisdeoxy-7,8-dihydrogenipin* (XXIVa). A solution of 109 mg. of 1,10-bisdeoxy-7,8-dihydrogenipin in 5 cc. of acetic acid consumed 1 equivalent of hydrogen after hydrogenation (room temperature, atmospheric pressure) for 20 hr. in the presence of 21 mg. of platinum oxide catalyst. The infrared spectrum of the resulting oil (IIIa) did not contain any more the 6.1 μ enol ether band. The ester was saponified by heating under reflux for 3 hr. with 0.5*N* ethanolic sodium hydroxide solution and the acid IIIb was converted directly at *p*H 6.5 (for details see above described reaction of XXIVb) into its *S*-benzylthiouronium salt. After recrystallization from hot water, the colorless needles possessed m.p. 138–141°, $[\alpha]_D$ +40° (*c*, 0.445 in methanol) and proved to be identical in all respects with the earlier reported[8] *S*-benzylthiouronium salt of the acid IIIb obtained (without isolation of intermediates) by successive hydrogenation of genipin (I) with palladium-charcoal in methanol and then platinum oxide in acetic acid solution.

Chromium trioxide oxidation of 10-deoxy-7,8-dihydrogenipin (IIa). To a solution of 1.90 g. of 10-deoxy-7,8-dihydrogenipin (IIa) in 5 cc. of acetone cooled to 10° was added dropwise 3.4 cc. of 8*N* chromium trioxide–sulfuric acid solution,[9] rapid reaction occurring only with the first 1.2 cc. of reagent. Water was added, the mixture was extracted with ether, and the product was then separated into a neutral (0.96 g., not extracted by bicarbonate) and an acidic (0.544 g., bicarbonate-soluble) fraction.

The neutral product was chromatographed on 100 g. of silica gel, fifty-nine 25-cc. fractions being collected. Fractions 13–20 were removed from the column with benzene and after distillation at 80°/0.01 mm. there was obtained 133 mg. of a colorless mobile oil corresponding to the lactone *1-dehydro-10-deoxy-7,8-dihydrogenipin* (IV), $\lambda_{max}^{CHCl_3}$ 5.64, 5.83, and 6.05 μ, $\lambda_{max}^{C_2H_5OH}$ 237 mμ, log ϵ 4.02, plain positive rotatory dispersion curve[10] ($[\alpha]_{589}$ +82°, $[\alpha]_{500}$ +130°, $[\alpha]_{400}$ +205°, $[\alpha]_{300}$ +530°, $[\alpha]_{280}$ +780°) in methanol solution (*c*, 0.16).

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71; OCH_3, 14.72; C—CH_3, 7.14. Found: C, 62.83; H, 6.74; OCH_3, 14.91; C—CH_3, 7.41.

From the more polar fractions of the chromatogram there was obtained a viscous yellowish oil, whose infrared spectrum was practically indistinguishable from that of 10-deoxy-7,8-dihydrogenipin (IIa).

The acid fraction (VIIa) was methylated with diazomethane and the methyl ester chromatographed carefully on neutral alumina (activity II). The portion eluted with benzene-chloroform was distilled in high vacuum to afford an oil, $\lambda_{max}^{CHCl_3}$ 5.75 μ, whose analysis was consistent with formulation VIIb.

Anal. Calcd. for $C_{11}H_{16}O_5$: C, 57.88; H, 7.07; O, 35.05. Found: C, 57.68; H, 7.13; O, 35.22.

1-Methyl-2-carboxycyclopentane-3-acetic acid (Va). 1-Dehydro-10-deoxy-7,8-dihydrogenipin (IV) (122 mg.) was left at room temperature for 20 hr. with 5 cc. of 10% sodium hydroxide solution and 1 cc. of ethanol. Dilution with water, acidification, extraction with ether, washing with bicarbonate solution, and acidification of the washes afforded 84 mg. of the dibasic acid Va, m.p. 99.5–104° after distillation at 150°/0.005 mm. Two recrystallizations from hexane ether yielded an analytical specimen, m.p. 99.5–105° (104.5–107.5° after drying for 3 days at 0.5 mm. over phosphorus pentoxide), $[\alpha]_D$ +20° (*c*, 0.21 in methanol), $\lambda_{max}^{CHCl_3}$ 5.83 μ and typical "acid" absorption in 3–4 μ region.

Anal. Calcd. for $C_9H_{14}O_4$: C, 58.05; H, 7.58; O, 34.37; C—CH_3, 8.08; mol. wt., 186.2. Found: C, 58.14; H, 7.38; O, 34.60; C—CH_3, 6.97; neut. equiv., 90.

The same acid Va was also obtained when the crude aldehydo acid VIIa was stirred overnight with aqueous 10% sodium hydroxide.

A 28-mg. sample of the dicarboxylic acid Va was methylated with diazomethane and the resulting dimethyl ester Vb distilled at 0.1 mm; $\lambda_{max}^{CHCl_3}$ 5.78 μ.

Anal. Calcd. for $C_{11}H_{18}O_4$: C, 61.66; H, 8.47. Found: C, 61.11; H, 8.20.

Lithium aluminum hydride reduction of 10-deoxy-7,8-dihydrogenipin (IIa). A solution of 7.01 g. of 10-deoxy-7,8-dihydrogenipin (IIa) in 100 cc. of anhydrous ether was added dropwise to a stirred suspension of 13 g. of lithium aluminum hydride in 300 cc. of dry ether. The mixture was stirred at room temperature for 17 hr., the excess reagent was destroyed with ethyl acetate, saturated aqueous sodium sulfate was added to precipitate inorganic salts, and the solution was then dried by addition of anhydrous sodium sulfate. The solution was decanted from the residue which was triturated with ether. The combined ether solutions were evaporated and the resulting 5.46 g. of colorless oil was chromatographed in ether-benzene (1:1) on 200 g. of Merck acid-washed alumina. The amount of ether was gradually increased up to 100% and then methanol was added to the ether in proportions of 2–5% collecting a total of 13 fractions. Fractions 3–4 (ether-benzene 7:3) yielded 0.32 g. of a colorless oil[11] which was distilled at a bath temperature of 60–70°/0.15 mm., $\lambda_{max}^{CHCl_3}$ 2.95 and 5.77 μ, $[\alpha]_D$ +22° (*c*, 1.09 in chloroform). It was not investigated further.

Anal. Calcd. for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66; O, 18.79; C—CH_3, 8.83. Found: C, 70.01; H, 10.14; O, 19.21; C—CH_3, 14.16; OCH_3, 0.0.

Fractions 9–10 (ether-methanol 98:2 and 95:5) gave 3.72 g. of colorless oil which could be crystallized at Dry Ice–acetone temperature from ether–petroleum ether[33]; yield, 1.30 g., m.p. 37–39°, $[\alpha]_D$ −9.7° (*c*, 1.34 in chloroform), $\lambda_{max}^{CHCl_3}$ 2.81, 3.00, 6.08, and 11.03 μ. The substance possesses structure VIIIa.

Anal. Calcd. for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66; O, 18.79; C—CH_3, 8.83. Found: C, 70.57; H, 10.58; O, 19.00; C—CH_3, 8.45.

The *acetate* VIIIb was prepared by leaving 823 mg. of VIIIa overnight with 15 cc. of pyridine and 2.9 g. of acetic anhydride. The product was isolated by ether extraction and was purified by chromatography on 46 g. of Merck

(33) Care had to be taken in the crystallization of this material in that it had to be filtered at Dry Ice temperature and then placed immediately into a vacuum desiccator until all traces of solvent had been removed. Failure to remove all solvent led to crystals which melted at room temperature.

acid-washed alumina, elution with benzene-ether (9:1 and 6:1) and distillation at a bath temperature of 130°/0.47 mm., $[\alpha]_D$ −4.6° (*c*, 1.52 in chloroform), $\lambda_{max}^{capill.}$ 5.71, 6.05, 11.02 μ.

Anal. Calcd. for $C_{14}H_{22}O_4$: C, 66.13; H, 8.72; O, 25.17; acetyl, 16.90. Found: C, 65.93; H, 8.69; O, 25.52; acetyl, 32.82.

Ozone was passed at −70° through a 1.003-g. sample of the acetate VIIIb dissolved in 25 cc. of methylene chloride until the solution turned blue. It was then poured into a suspension of 4.0 g. of zinc dust in 15 cc. of acetic acid and the mixture was stirred at room temperature for 3 hr. The zinc was filtered and the filtrate was neutralized with 2% sodium hydroxide solution and extracted with ether. Washing, drying and evaporation of the ether left 0.947 g. of liquid which was chromatographed in benzene solution on 35 g. of Merck's acid-washed alumina. Elution with benzene-ether (95:5) and crystallization from ether–petroleum ether at −70° afforded 0.53 g. of the *keto diacetate* IX, m.p. 35–36°, $\lambda_{max}^{CHCl_3}$ 5.77 μ, positive rotatory dispersion Cotton effect[10] in methanol solution (*c*, 0.095): $[\alpha]_{589}$ +88°, $[\alpha]_{302.4}$ +1078°, $[\alpha]_{260}$ −676°, $[\alpha]_{252.5}$ −613°. This was changed only slightly upon the addition of 0.1*N* sodium hydroxide: $[\alpha]_{302.5}$ +986°, $[\alpha]_{260}$ −756°.

Anal. Calcd. for $C_{13}H_{20}O_5$: C, 60.91; H, 7.87; O, 31.24; C—CH_3, 5.86; acetyl, 16.79. Found: C, 60.92; H, 7.90; O, 31.52; C—CH_3, 17.20; acetyl, 35.68.

1-Methyl-3-isopropylcyclopentane-2-carboxylic acid (Xb). The diol VIIIa (350 mg.) was hydrogenated in methanol solution at room temperature and atmospheric pressure in the presence of 0.3 g. of prehydrogenated 10% palladized charcoal catalyst, hydrogen uptake corresponding to 2 equivalents having ceased within 2 hr. Filtration of the catalyst and distillation at 3 mm. yielded *1-methyl-2-hydroxymethyl-3-isopropylcyclopentane* (Xa) as a sweet-smelling colorless liquid, which appeared to be homogeneous by vapor phase chromatography at 175° using a 10 ft. column of silicone–fire brick (1:3). The infrared bands at 6.05 and 11.02 μ corresponding to the terminal olefin grouping had disappeared, but peaks at 7.22 and 7.31 μ ascribable to the *gem*-dimethyl grouping could be noted.

Anal. Calcd. for $C_{10}H_{20}O$: C, 76.86; H, 12.90. Found: C, 76.34; H, 12.59.

A solution of the above alcohol Xa (250 mg.) in 5 cc. of acetic acid was oxidized (room temperature, 20 hr.) with 0.2 g. of chromium trioxide in 5 cc. of 90% acetic acid. Dilution with water, extraction with ether, washing with 5% sodium carbonate solution, acidification, and re-extraction with ether led to 0.162 g. of the acid Xb, which was transformed into its *S-benzylthiouronium* salt by the procedure described earlier for the acid XXIVb. Recrystallization from aqueous methanol provided 0.215 g. of the pure salt, m.p. 121–122°, $[\alpha]_D$ −5.5° (*c*, 1.09 in methanol), which exhibited a plain, negative dispersion curve[10] in methanol dropping to $[\alpha]_{272.5}$ −100°.

Anal. Calcd. for $C_{18}H_{28}N_2O_2S$: C, 64.25; H, 8.39; N, 8.33; O, 9.51; S, 9.53. Found: C, 64.17; H, 7.97; N, 8.31; O, 9.78; S, 9.49.

The salt (212 mg.) was dissolved in 30 cc. of 70% aqueous methanol and passed through a column (25 × 1 cm.) of Dowex-50 (pretreated with 2% aqueous sodium hydroxide, 5% aqueous hydrochloric acid, water, and finally 70% aqueous methanol). The eluate was made slightly alkaline by the addition of a few drops of 2% sodium hydroxide solution and most of the methanol was then removed *in vacuo*. The solution was then acidified, extracted with ether, washed, dried, and evaporated to afford 102 mg. of the *acid* Xb, which was distilled at a bath temperature of 80°/0.12 mm., $\lambda_{max}^{capill.}$ 5.88, 7.23, and 7.33 μ. The acid exhibited a plain positive rotatory dispersion curve in methanol solution (*c*, 0.205) which however commenced on the negative side in the visible: $[\alpha]_{700}$ −3°, $[\alpha]_{589}$ −7°, $[\alpha]_{300}$ 0°, $[\alpha]_{257.5}$ +126°.

Anal. Calcd. for $C_{10}H_{18}O_2$: C, 70.54; H, 10.66; O, 18.80; C—CH_3, 8.83; mol. wt., 170. Found: C, 70.75; H, 10.21; O, 18.84; C—CH_3, 12.23; neut. equiv., 176.

Methylation with diazomethane and distillation at a bath temperature of 50°/0.55 mm. led to the *methyl ester* Xc, whose rotatory dispersion curve in methanol (*c*, 0.26) was similar to that of the acid Xb: $[\alpha]_{700}$ −1°, $[\alpha]_{589}$ −6°, $[\alpha]_{275}$ +87°.

Anal. Calcd. for $C_{11}H_{20}O_2$: C, 71.69; H, 10.94; O, 17.37; OCH_3, 16.84. Found: C, 71.06; H, 10.76; O, 17.38; OCH_3, 16.61.

Hydrogenation of nepetalactone (XI). Freshly distilled nepetalactone (XI) (3.196 g.) was hydrogenated in 50 cc. of acetic acid with 0.5 g. of prereduced platinum oxide catalyst as described by Meinwalc.[16] Hydrogen uptake ceased after 20 hr. (1.8 equivalents of hydrogen) and the usual work-up gave 2.435 g. of the oily acid XII,[16] which was directly transformed into its *S*-benzylthiouronium salt (4.52 g.), m.p. 118–119° (after recrystallization from aqueous methanol). The infrared spectrum of this salt was practically identical with that of the corresponding salt of the isomeric acid Xb, but a definite difference was noted in their rotations, the *S*-benzylthiouronium salt of XII exhibiting $[\alpha]_D$ +2.8° (*c*, 1.07 in methanol) and its rotatory dispersion curve being of the plain positive type[10] (rising to $[\alpha]_{260}$ +49°).

Anal. Calcd. for $C_{18}H_{28}N_2O_2S$: C, 64.25; H, 8.39; N, 8.33; S, 9.53. Found: C, 64.23; H, 8.22; N, 8.34; S, 9.30.

A portion of this salt was decomposed on Dowex-50 resin and the liberated acid XII was distilled at a bath temperature of 80°/0.44 mm. Its optical rotatory dispersion in methanol (*c*, 0.20) was characterized by a plain negative curve: $[\alpha]_{700}$ −10°, $[\alpha]_{589}$ −12°, $[\alpha]_{260}$ −178° and its infrared spectrum (chloroform or liquid film) was quite distinct from that of the acid Xb.

Anal. Calcd. for $C_{10}H_{18}O_2$: C, 70.54; H, 10.66; O, 18.80; mol. wt., 170. Found: C, 70.30; H, 10.59; O, 18.94; neut. equiv., 175.

Ozonolysis of 10-deoxy-7,8-dihydrogenipin (IIa) *to cis,cis-nepetic acid* (XIIIa). 10-Deoxy-7,8-dihydrogenipin (IIa) (1.638 g.) was ozonized in 10 cc. of methylene chloride at −70° until the solution turned blue (approximately 30 min.). The solution was then added dropwise to 15 cc. of 5% aqueous sodium hydroxide containing 6 cc. of 30% hydrogen peroxide, stirred for 30 min. and the methylene chloride was then removed *in vacuo* at room temperature. An additional 10 cc. of 30% hydrogen peroxide was added and the mixture was stirred overnight. Acidification with hydrochloric acid, addition of solid sodium sulfite, salting out with sodium chloride, and extraction with ether, followed by washing with sodium chloride solution, drying, and evaporation afforded 0.868 g. of crystals, m.p. 90–110°. These were suspended in water, made alkaline to phenolphthalein with aqueous barium hydroxide and the *barium salt* of *cis, cis*-nepetic acid (XIIIa) was filtered. The salt was decomposed with 5% hydrochloric acid, the acid was extracted with ether and recrystallized once from ether–petroleum ether, whereupon 0.518 g. of pure *cis, cis*-nepetic acid (XIIIa) was isolated, m.p. 136–137°, $[\alpha]_D$ −0.9° (*c*, 1.05 in chloroform), plain positive rotatory dispersion curve in methanol solution (*c*, 0.56): $[\alpha]_{700}$ +31°, $[\alpha]_{589}$ +37°, $[\alpha]_{500}$ +51°, $[\alpha]_{400}$ +107°, $[\alpha]_{300}$ +299°, $[\alpha]_{255}$ +774°. The infrared spectrum of this acid in chloroform solution was completely identical with that of racemic *cis, cis*-nepetic acid.[18, 21]

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.80; H, 7.03; O, 37.17; C—CH_3, 8.73; mol. wt., 172. Found: C, 55.93; H, 6.96; O, 37.39; C—CH_3, 7.87; neut. equiv., 92.

Small samples of the optically active and racemic acids were methylated with diazomethane and the methyl esters (XIIIb) distilled at a bath temperature of 70°/0.2 mm. Their respective infrared spectra (liquid film) were completely identical.

(−)-*trans, cis-Nepetic acid* (XVIIIa). The *cis, cis*-nepetic acid XIIIa (0.976 g.) was heated under reflux for 6 hr. with

5.5 cc. of acetic anhydride, the excess acetic acid and acetic anhydride were then removed *in vacuo*, and the residual nepetic acid anhydride was evaporatively distilled at 0.05 mm. The crystalline distillate was immediately heated under reflux for 2 hr. with 6 cc. of anhydrous methanol, a solution of 0.5 g. of sodium metal in 10 cc. of anhydrous methanol was added and heating was continued for 30 min. Water (5 cc.) was now added and the reaction mixture was heated under reflux for a further 1 hr. in order to saponify the half-ester XVIIIb. The methanol was distilled off under reduced pressure and the solution was evaporated repeatedly with water. The residue was dissolved in water, acidified, and the crude acid obtained after ether extraction was converted into its barium salt. This proved to be water-soluble, thus indicating the absence of any *cis, cis* isomer XIIIa (insoluble barium salt) and after acidification and repeated ether extraction, there was obtained (after several recrystallizations from ether–petroleum ether) 0.546 g. of (−)-*trans, cis*-nepetic acid (XVIIIa), m.p. 95–100° (Kofler block), 98–100° (capillary), $[\alpha]_D$ −66.2° (*c*, 1.08 in chloroform). The melting point remained unchanged after countercurrent distribution (ether *vs.* phosphate-citrate buffer of *p*H 6.05) or silica gel chromatography.

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.80; H, 7.03; O, 37.17. Found: C, 55.71; H, 7.16; O, 37.01.

Lithium aluminum hydride reduction of 1,10-anhydro-7,8-dihydrogenipin (XXVII). A solution of 5.203 g. of 1,10-anhydro-7,8-dihydrogenipin (XXVII) in 70 cc. of anhydrous ether was reduced with 9.6 g. of lithium aluminum hydride exactly as described above for the analogous reduction of IIa. The crude product (4.623 g.) was chromatographed in benzene solution on 150 g. of Merck acid-washed alumina to afford in the ether-methanol (9:1) eluates 2.09 g. of the *unsaturated aldehyde* XXVIII. The analytical samples was distilled at 120°/0.05 mm. and exhibited $[\alpha]_D$ +40.3° (*c*, 1.34 in chloroform), $\lambda_{max}^{C_2H_5OH}$ 216 mμ, log ε 3.73, $0_{max}^{CHCl_3}$ 2.77, 2.92, 3.69 (w), 5.92 (s), and 6.03 (w) μ.

Anal. Calcd. for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74. Found: C, 65.36; H, 7.35.

Treatment of a sample of the aldehyde XXVIII with 2,4-dinitrophenylhydrazine in methanolic hydrochloric acid for 30 min. at room temperature led to the *2,4-dinitrophenylhydrazone* XXX, which was recrystallized from methanol-chloroform; m.p. 182–184°, $\lambda_{max}^{CHCl_3}$ 366 mμ, log ε 4.42.

Anal. Calcd. for $C_{17}H_{22}N_4O_6$: C, 53.96; H, 5.86; N, 14.81; O, 25.37; OCH_3, 8.20. Found: C, 54.16; H, 5.59; N, 14.72; O, 25.59; OCH_3, 8.04.

The ether-methanol (7:3) eluates of the original chromatogram furnished 0.303 g. of the *triol* XXIXa as a sticky oil, which was distilled at 150°/0.03 mm, $\lambda_{max}^{CHCl_3}$ 2.90, 6.13 (w) μ. For purposes of characterization, the triol was acetylated by heating under reflux for 6 hr. with acetic anhydride–pyridine, evaporating to dryness and extraction with ether. The crude *triacetate* XXIXb was purified by chromatography on 12 g. of Merck acid-washed alumina, elution with benzene-ether (9:1 and 6:1) and finally distillation at 120°/0.05 mm.; $[\alpha]_D$ +25° (*c*, 1.00 in chloroform), $\lambda_{max}^{CHCl_3}$ 5.79 (s), 6.06 (w), 8–8.4 μ and 11.06 μ.

Anal. Calcd. for $C_{16}H_{24}O_6$: C, 61.52; H, 7.75; O, 30.73. Found: C, 61.89; H, 7.84; O, 30.32.

Reduction of 0.547 g. of the unsaturated aldehyde XXVIII with 1.4 g. of lithium aluminum hydride followed by acetylation of the resulting triol produced 0.44 g. of the triacetate XXIXb.

STANFORD, CALIF.

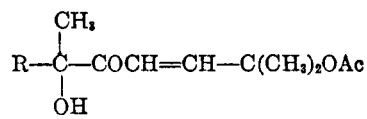
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STANFORD UNIVERSITY]

Reduction and Oxidation Products of Cucurbitacin B[1]

W. SCHLEGEL, A. MELERA, AND C. R. NOLLER

Received May 13, 1960

Catalytic hydrogenation of cucurbitacin B yields two products, a dihydro derivative and a dihydrodeacetoxy derivative. Reaction with zinc dust gives a deacetoxy derivative with migration of the double bond out of conjugation with a carbonyl group. Oxidation of acetylated cucurbitacin B with chromic acid gives β,β-dimethylacrylic acid and two methyl ketones. These results can be accommodated by a side chain having the structure



Cucurbitacin B, present in numerous members of the *Cucurbitaceae*,[2] is one of the bitter principles isolated from *Echinocystis fabacea*.[3] A second product, called fabacein, also was isolated, separation being accomplished by fractional crystallization. Since then it has been found that the separations can be made readily by the use of a chromatographic column in which the immobile phase is formamide on Celite (4:5 by weight) and the elutant is benzene or benzene–ethyl actate, thus providing a means of obtaining these substances in relatively large amounts with little difficulty. The present paper reports the details of a number of experiments on cucurbitacin B.

Cucurbitacin B (I) contains an acetoxy group, an α,β-unsaturated carbonyl group, two additional unconjugated carbonyl groups and three hydroxyl groups.[3,4] A determination of the molecular weight

(1) The results of some of these investigations were published in a preliminary report, *J. Org. Chem.*, 24, 291 (1959).

(2) P. R. Enslin, *J. Sci. Food Agri.*, 5, 411 (1954); S. Rehm, P. R. Enslin, A. D. J. Meeuse, and J. H. Wessels, *J. Sci. Food. Agr.*, 8, 679 (1957).

(3) W. O. Eisenhut and C. R. Noller, *J. Org. Chem.*, 23, 1984 (1958).

(4) P. R. Enslin, S. Rehm, and D. E. A. Rivett, *J. Sci. Food Agri.*, 8, 674 (1957).