Tetrahedron, 1965, Vol. 21, pp. 75 to 87. Pergamon Press Ltd. Printed in Northern Ireland

# CITRUS BITTER PRINCIPLES—II

## APPLICATION OF NMR TO STRUCTURAL AND STEREOCHEMICAL PROBLEMS

D. L. DREYER
Fruit and Vegetable Chemistry Laboratory,[1] Pasadena, California

(*Received* 3 *June* 1964; *in revised form* 25 *August* 1964)

Abstract—The NMR spectra of limonin, its congeners, and some of their derivatives are discussed. The results confirm the structures assigned to citrolin, limonexic acid, and many other degradation products of limonin. Stereochemical assignments are made to the 1-position in nomilin and the 17-position in obacunone on the basis of their NMR spectra. This furfurylic proton at $C_{17}$ in limonin is compared with a similar proton in columbin. Evidence for an anisotropic shielding effect of the epoxy group is discussed.

ALTHOUGH NMR studies have been important in the chemical structure determination of limonin (I),[2] only very meager data have been published, for evaluation, largely on the C-methyl[2] and the furan resonances.[3]

Some of the conversion products of limonin involve deep-seated changes in structure or novel reactions. Among these are limonilic acid (II), citrolin (III), deoxylimonic acid (IVa), isohexahydrolimoninic acid (V), and limonin diosphenol (VI). NMR data would aid in confirming the structures of many of these degradation products of limonin and its congeners.[4,5] In addition, a catalog of NMR data would be of potential aid in the structure determination of many limonoid bitter principles of yet unassigned structure occurring in the Rutaceae and Meliaceae.[6]

The arrangement of a relatively large number of functional groups in a framework of rigid stereochemistry causes the protons to be shielded to different extents. Thus, many of the protons appear in distinctive regions of the spectrum and there are few overlapping bands. The location of functional groups that exhibit diamagnetic anisotropy on a rigid steroid framework would induce fields which can change the chemical shifts of neighboring protons in accord with their individual orientation to the functional group. These secondary fields can then be evaluated in a series of compounds where regular changes in structure have been made.

In many cases where overlapping bands did occur they could be separated or shifted by taking the spectra in different solvents. Band shapes and coupling constants

---

[1] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

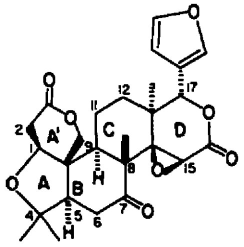
[2] D. Arigoni, D. H. R. Barton, E. J. Corey, O. Jeger, L. Caglioti, Sukh Dev, P. G. Ferrini, E. R. Glazier, A. Melera, S. K. Pradhan, K. Schaffner, S. Sternhell, J. F. Templeton and S. Tobinaga, *Experientia* **16**, 41 (1960).

[3] E. J. Corey, G. Slomp, Sukh Dev, S. Tobinaga and E. R. Glazier, *J. Amer. Chem. Soc.* **80**, 1204 (1958).

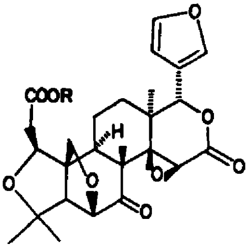
[4] D. H. R. Barton, S. K. Pradhan, S. Sternhell and J. F. Templeton, *J. Chem. Soc.* 255 (1961).

[5] A. Melera, K. Schaffner, D. Arigoni and O. Jeger, *Helv. Chim. Acta* **40**, 1420 (1957).

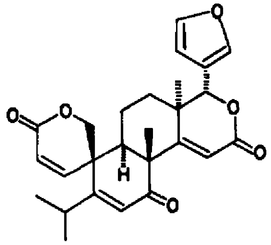
[6] Unpublished results from these laboratories. See also C. W. L. Beven, A. H. Rees and D. A. H. Taylor, *J. Chem. Soc.* 983 (1963), and previous papers in this series.



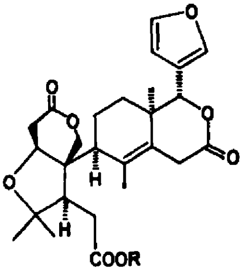
I



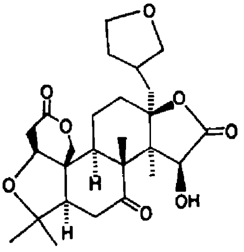
II a R = H
II b R = $CH_3$



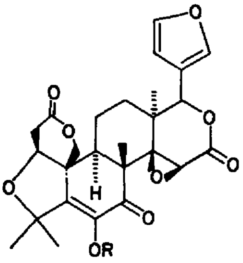
III



IV a R = H
IV b R = $CH_3$



V



VI a R = H
VI b R = Ac

could thus be established with certainty. Deuteriochloroform-dimethyl sulfoxide (DMSO), deuteriochloroform-acetonitrile, and trifluoroacetic acid were most useful in this respect.

Some of the higher melting limonin derivatives were not sufficiently soluble in chloroform for good NMR spectra. They would usually dissolve sufficiently in 10–20% dimethyl sulfoxide in chloroform. Trifluoracetic acid also proved to be a good solvent for limonin derivatives; however, the solutions colored slightly upon standing several days.

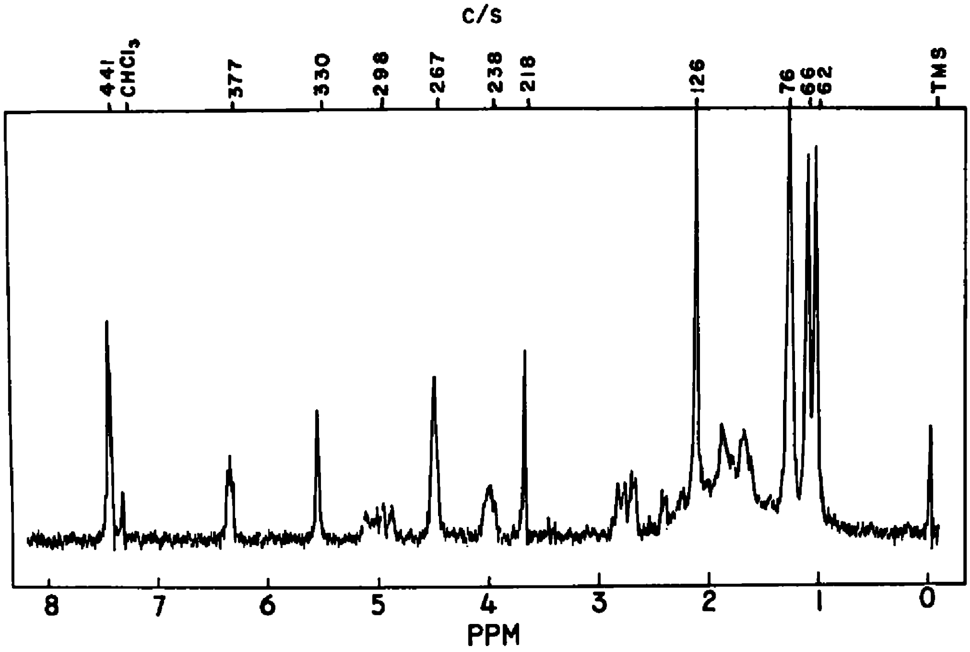


FIG. 1. NMR spectrum of epilimonyl acetate in deuteriochloroform

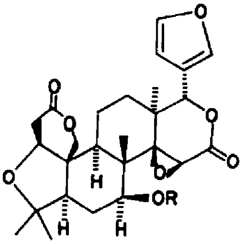
The gross features of the NMR spectra for a typical example are illustrated with epilimonyl acetate (VIIb) in Fig. 1. The general features of the NMR spectra of limonin (I) and most of its derivatives starting from the high field end of the spectrum can be summarized as follows:

1. Bands due to the C-methyl resonances.
2. The absorption bands for the four protons at $C_2$ and $C_6$, α- to the carbonyl groups. For most limonin derivatives these bands were not interpretable.
3. A sharp singlet due to the proton at $C_{15}$, adjacent to the epoxy group. This band sometimes overlapped the band due to the proton on $C_1$.
4. A broad band due to the proton on $C_1$ which in some cases was resolved into a symmetrical triplet.
5. An AB system from the protons on $C_{19}$, which in several derivatives collapsed into a broadened singlet.
6. A very slightly broadened singlet caused by the proton at $C_{17}$, adjacent to the furan ring.

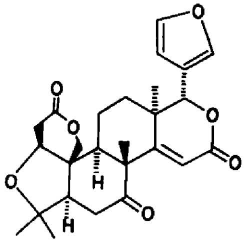
7. The furan absorptions, which have been discussed previously.[3] Structural changes in the D-ring greatly influenced the multiplicity of the furan bands.

The resonances, with their observed coupling constants, of the 24 substances examined in this study are given in Table 1. All bands had areas consistent with their assignments.[7]

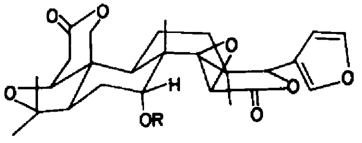
In most derivatives, structurally similar to limonin, the C-methyl resonance furthest downfield is that due to the C-methyl at $C_{13}$. The downfield shift of this methyl group is presumably due to the anisotropic effect of the furan ring. Since the *gem*-dimethyls at $C_4$ are attached to a carbon atom which is in turn attached to an ether oxygen, the chemical shift of such a group (70–80 c/s)[8] would be further downfield than that observed for the C-methyl at $C_8$, which occurs at 65 c/s in limonin itself. Thus, the assignment of the highfield C-methyl peak falls to the C-methyl group at $C_8$ largely by elimination. The pattern of shifts with changes in structure observed for the $C_8$ resonance is consistent with this assignment. For example, this resonance shifts downfield in deoxylimonin (VIII) but upfield in limonin oxime (IX), limonol (Xa) and epilimonol (VIIa). The downfield C-methyls in limonin diosphenol (VIa and VIb) must be the *gem*-dimethyls at $C_4$.



VII a R = H
VII b R = Ac



VIII



X a R = H
X b R = Ac

One of the C-methyl resonances in the limonilic acid (II, XVI, XVIII) derivatives shifts strikingly downfield to ca. 100 c/s. This shift must be due to one of the C-methyl groups at the 4- or 8-positions which are located 1,3-diaxially to the new ether oxygen

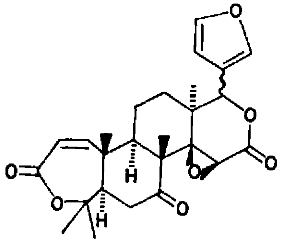
---

[7] Dreiding models were found to be most helpful in the interpretation of the spectra.

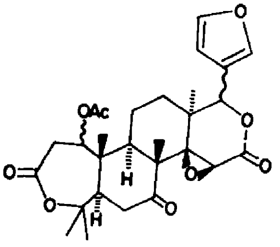
[8] For examples see N. S. Bhacca, L. F. Johnson, J. N. Shoolery, Spectrum No. 280, *NMR Spectra Catalog*, Varian Associates, Palo Alto, California; S. Itô, M. Kodama, T. Nozoe, H. Hikino, Y. Hikino, Y. Takeshita and T. Takemoto, *Tetrahedron Letters* 1787 (1963).

at $C_6$. Resonances due to angular methyl groups located 1,3-diaxially to single bonded oxygen functions are known to shift significantly downfield.[9] However, only *one* C-methyl band shifts downfield in the limonilic acid derivatives. It appears to be the methyl group on $C_8$. Rupture of the A′-ring allows the *gem*-dimethyl group at $C_4$ on the A-ring to fold back somewhat to relieve 1,3-interactions with the $C_{19}$ methylene group whereas the $C_8$ methyl is forced even closer to the ether bridge oxygen as a consequence of its formation.

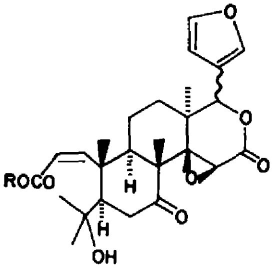
Obacunone (XI) has three and nomilin (XII) has two C-methyls displaced rather far downfield to 87–93 c/s. The other obacunone derivatives XIIIa and XIIIb with the A-ring opened have only one C-methyl in this downfield region. On this basis one of these downfield bands is assigned to the $C_{19}$ methyl while the others are assigned to the C-methyls at $C_4$. In the obacunone case both *gem*-dimethyls are subjected to anisotropic influences of the carbonyl and double bond, whereas only one of the *gem*-dimethyls in nomilin is so influenced. In the ring opened derivatives, XIIIa and XIIIb, the rigid A-ring stereochemistry is lost and the *gem*-dimethyl group is no longer in close proximity to the α,β-unsaturated carbonyl system. Thus only the $C_{19}$ resonance occurs downfield.



XI

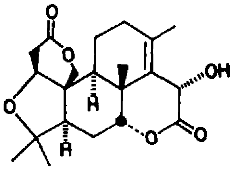


XII



XIII a R = H

XIII b R = $CH_3$



XIV

---

[9] K. Kuriyama, E. Kondo and K. Tori, *Tetrahedron Letters* 1485 (1963); Y. Kawazoe, Y. Sato, H. Natsume, H. Hasegawa, T. Okamoto and K. Tsuda, *Chem. & Pharm. Bull. Japan* **10**, 338 (1962); Y. Kawazoe, Y. Sato, T. Okamoto and K. Tsuda, *Ibid.* **11**, 328 (1963); T. Okamoto and Y. Kawazoe *Ibid.* **11**, 643 (1963).

The NMR spectra of merolimonol (XIV) and several of its derivatives have been discussed previously.[3,10] The only additional feature worthy of comment is the absorption centered at ca. 165 c/s due to the protons on $C_2$, α- to the carbonyl. This is the AB part of an ABX pattern. Six of the eight lines expected for such a pattern[11] are clearly discernible in all of the merolimonol derivatives examined.[12] Only the two upfield, low intensity lines are overlapped by other bands. Limonyl (Xb) and epilimonyl (VIIb) acetates clearly show a pattern identical to that of the merolimonol derivatives in the region of 165 c/s (Fig. 1). These bands are obscured in most other limonin derivatives by the protons at $C_6$, α- to the keto group.

The proton at $C_{15}$ on the epoxy group caused a characteristically sharp singlet with good ringing whose chemical shift was to much lower field than expected for such a group.[13] Moreover, the position of this band depended upon the nature of the group at $C_7$. It shifted upfield when the keto was reduced as in limonyl (Xb) and epilimonyl (VIIb) acetates or converted to the oxime (IX). The $C_{15}$ proton is clearly influenced by the anisotropic effect of the $C_7$ keto carbonyl and must be in an area of negative shielding by the keto group in order to account for the observed downfield shift.

Epilimonol (VIIa) is a striking exception to this pattern of shifts for the $C_{15}$ epoxy resonance. The epoxy resonance in this case occurs at 273 c/s; a 40 c/s downfield shift away from limonol (Xa). An equatorial hydroxyl group on $C_7$ is located especially close to the $C_{15}$ proton as in epilimonol (VIIa). The $C_{15}$ proton is thus strongly deshielded by fields associated with the anisotropies of the carbon-oxygen bond. The effect does not appear at all with epilimonyl acetate (VIIb) or the other $C_7$ isomer, limonol (Xa) where, due to the stereochemistry at $C_7$ the axial hydroxyl group is not in close proximity to the $C_{15}$ proton.

Whereas the position of the $C_{15}$ epoxy resonances of obacunone (XI), nomilin (XII), and their derivatives (XIIIa, XIIIb; ca. 220 c/s) are downfield from the range normally accepted for such protons[13] (175–200 c/s), it does not approach that of the epoxy proton of the structurally similar limonin derivatives, ca. 240 c/s. The difference between the obacunone (XI) case and limonin (I) must arise from conformational differences between their A-rings. Structural changes in the A-ring cause alterations in the B-ring conformation, slightly altering the position of the $C_7$ keto group. In obacunone (XI) and obacunoic acid derivatives (XIIIa, XIIIb), the B-ring is in an undistorted chair conformation. The fusion of a 5-membered ring onto the B-ring which occurs in forming iso-obacunoic acid or the limonin derivatives causes flattening of the B-ring somewhat which in turn changes the angle of the 7-keto group. With flattening of the B-ring, the 7-keto group will be more in plane with the $C_{15}$ epoxy proton. Consequently the $C_{15}$ proton of obaucnone (XI) resonates 55 c/s upfield from the $C_{15}$ proton of iso-obacunoic acid (XV).

The dependence of the position of the $C_{15}$ resonance on the $C_7$ group is very pronounced in the case of methyl limonilate oxime (XVI), where the $C_{15}$ epoxy proton

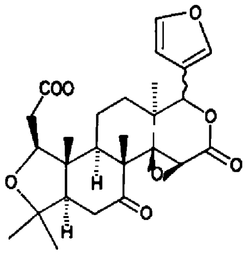
[10] D. L. Dreyer, *Experientia* **20**, 297 (1964).

[11] H. Conroy, *Advances in Organic Chemistry* Vol. 2; p. 304. Interscience, New York (1960).

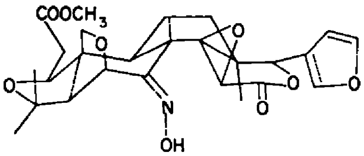
[12] An exceptionally large coupling constant of 17 c/s was observed for the AB resonances of the ABX system. Such large coupling constants are characteristic of the methylene groups α- to carbonyl groups; T. Takahashi, *Tetrahedron Letters* 565 (1964).

[13] For example, the epoxy proton in ethyl α,β-epoxycyclohexylidene acetate occurs at 187 c/s and in isophorone oxide at 176 c/s.

occurs at an extreme downfield value of 306 c/s. The assignmnent of the $C_{15}$ band in this case rests primarily on its excellent ringing. The B-ring in methyl limonilate (XVI) oxime is held rigidly in an undistorted chair conformation due to the ether bridge as compared to limonin oxime (IX). A 64 c/s downfield shift of the $C_{15}$ band results from the angle change at $C_7$.



XV



XVI

Zürcher[14] has suggested a similar explanation to account for shifts in the resonance of the $C_{19}$ methyl with introduction of substituents in the C- or D-rings of 11-keto-steroids. In the steroid case, substitution in certain locations can cause flattening of the C-ring somewhat and change the position of the 11-keto group with respect to the $C_{19}$ methyl. The $C_{19}$ methyl will then be subjected to slightly different anisotropic fields with various degrees of distortion of the C-ring from a true chair form.

The resonance of an equatorial proton is usually further downfield than that due to an axial proton by about 30–35 c/s.[15,16] However, this order is reversed in the case of epilimonol (Xa), limonol (VIIa), and their acetates. X-ray studies on an epilimonyl derivative[17] have shown that the B-ring is in a chair conformation and the acetoxy group is equatorial. Jefferies *et al.*[18] have recently discussed long range shielding by an epoxy group in beyerol derivatives. The $H_7$ proton in limonyl acetate (Xb) is equatorial and is located on the face of the epoxide ring. Because of the direction of shift of its resonance, upfield from its normal position, the $H_7$ proton must be in a region of positive shielding. Thus, long range shielding by the epoxide group accounts for the inverted order of absorption by the $H_7$ protons in limonyl and epilimonyl acetates. According to Tori *et al.*[19] the anisotropic effect of an epoxy ring is due to both a ring current and the anisotropies associated with the carbon-oxygen bonds. This will affect the chemical shift of the relevant proton depending upon its position with respect to the epoxide ring.

Models indicate that the epoxide group should have a similar anisotropic effect on the resonance of the $C_{17}$ furfurylic proton. The absorption of the $C_{17}$ proton shifts upfield by 20–30 c/s in the deoxy derivatives. However, complete evaluation of the effect at the 17-position is prevented by the lack of the other 17-isomer with an equatorial proton.

[14] R. F. Zürcher, *Helv. Chim. Acta* **44**, 1380 (1961).

[15] Ref. 11, p. 280.

[16] For exceptions see A. Nickson, M. A. Castle, R. Harada, C. E. Berkoff and R. O. Williams, *J. Amer. Chem. Soc.* **85**, 2185 (1963), and Refs cited therein.

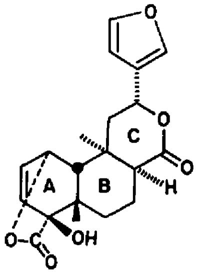
[17] S. Arnott, A. W. Davie, J. M. Robertson, G. A. Sim and D. G. Watson, *Experientia* **16**, 49 (1960).

[18] P. R. Jefferies, R. S. Rosich and D. E. White, *Tetrahedron Letters* 1853 (1963).

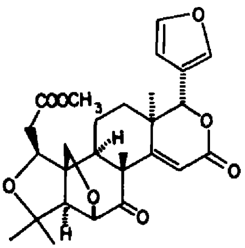
[19] K. Tori, K. Kitahonoki, Y. Takano, H. Tanida and T. Tsuji, *Tetrahedron Letters* 559 (1964).

Columbin (XVII),[20] a C-20 bitter principle, contains a furfurylic proton structurally similar to that found in the limonoids. On the basis of data summarized by Mathieson[21] the lactone ring (ring C) in XVII would be expected to be in a boat conformation. The resonance for the furfurylic proton in XVII has a quartet splitting pattern typical for an axial proton with adjacent axial and equatorial protons ($J_{a,a}$ 11, $J_{a,e}$ 5 c/s).[22] This would indicate that the furan ring is in an α-orientation. On the other hand, accepting an α-orientation of the furan ring, the NMR splitting pattern confirms the boat conformation of the lactone ring. The α-orientation of the furan ring in columbin was based previously upon application of a modification of the Hudson lactone rule.[23] The chemical shift of the furfurylic resonance in columbin (XVII) is strikingly close to the chemical shift of $H_{17}$ in limonin, 327 c/s.

The AB system of the methylene group on $C_{19}$ does not present any exceptional features. The chemical shifts and coupling constants (12–14 c/s) were consistent with much data reported on 19-acetoxysteroids.[24–26] The coupling constant dropped to about 8 c/s as a consequence of closing the 5-membered ether bridge in the limonilic acid (II) derivatives. 6β,19-Oxidosteroids show similar values.[25] Such AB systems occasionally collapse to a singlet with changes in neighbouring functional groups.[27] Such is the case with the diosphenol (VI) and limonol (Xa) among others.



XVII



XVIII

The furan resonances in all the deoxylimonin derivatives (IV, VIII, XVIII) showed a much more complex splitting pattern than those limonin derivatives having the epoxide group. Clearly the two α-furan protons have become much less equivalent in the case of the deoxy derivatives. The reason for this non-equivalence is not readily apparent. It could be due either to conformational differences in the C- or D-rings or to an anisotropic effect from the 14–15 double bond.

Limonin (I) has been chemically related to obacunone (XI)[28] through a route which correlates the stereochemistry at all positions except that at $C_{17}$. The range of the $H_{17}$ furfurylic resonance (327–334 c/s) in those limonin derivatives in which the

---

[20] D. H. R. Barton and D. Elad, *J. Chem. Soc.* 2085, 2090 (1956).

[21] A. McL. Mathieson, *Tetrahedron Letters* 81 (1963).

[22] Ref. 11, p. 310–311.

[23] K. H. Overton, N. G. Weir and A. Wylie, *Proc. Chem. Soc.* 211 (1961).

[24] J. Tadanier, *J. Org. Chem.* **28**, 1744 (1963).

[25] J. F. Bagli, P. F. Morand and R. Gaudry, *J. Org. Chem.* **28**, 1207 (1963).

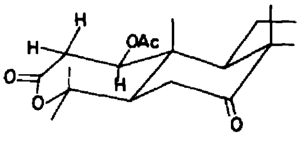
[26] T. Takahashi, *Agri. and Biol. Chem.* **27**, 633 (1963).

[27] A. Gaudemer, J. Polonsky and E. Wenkert, *Bull. Soc. Chim. Fr.* 407 (1964).

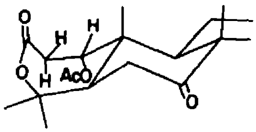
[28] T. Kubota, T. Matsuura, T. Tokoroyama, T. Kamikawa and T. Matsumoto, *Tetrahedron Letters* 325 (1961).

B-, C-, and D-rings are identical with obacunone, is very close to the range of the same band in all the obacunone derivatives (322-331 c/s). Indeed, in all the obacunone derivatives this band is within 5 c/s of the same band in limonin. This similarity suggests that the stereochemistry at the $C_{17}$ position is identical in the two series of compounds. The position of the $H_{17}$ band would be expected to be very sensitive to changes in stereochemistry at $C_{17}$, especially on the basis of the above arguments for an anisotropic effect from an epoxide ring, or, as the data collected in Table 1 shows, to chemical changes in the D-ring.

There has been no previous evidence bearing on the orientation of the acetoxy group at $C_1$ in nomilin (XII). The NMR spectrum of nomilin indicates that the acetoxy group is probably in the $\beta$-configuration. The splitting pattern of the band for $H_1$ was a quartet having a small coupling constant (2·5 c/s) with one of the neighboring protons on $C_2$ and a large coupling constant (6 c/s) with the other proton on $C_2$. This is a typical splitting pattern for one adjacent axial proton and one adjacent equatorial proton. Assuming planarity of the lactone group[21] there are only two possible conformations of the A-ring lactone in nomilin (XII). These are boat and chair conformations. Models indicate that these two conformations are of about equal energy. The $C_1$ splitting pattern would predict a $\beta$-acetoxy group for the chair conformation and an $\alpha$-acetoxy group for the boat conformation. The boat conformation seems unlikely, however, since the $C_{19}$ methyl group would be directly in a region of positive shielding by the lactone carbonyl. Since none of the C-methyl groups have shifted upfield a chair conformation is indicated and the acetoxy group must be in the $\beta$-position.



Chair



Boat

The NMR spectrum of citrolin (III) completely confirms the structure advanced by Barton *et al.*[4] on the basis of limited chemical and spectroscopic evidence. A typical AB doublet occurred in the vinyl region for the vinyl protons of the unsubstituted $\alpha,\beta$-unsaturated lactone in the A-ring. Sharp singlets occurred for the $\alpha$-vinyl protons of the $\alpha,\beta$-unsaturated keto group and the $\alpha,\beta$-unsaturated lactone of the D-ring. The vinyl resonance for the proton on $C_{15}$ is farther downfield than one would normally expect for an $\alpha,\beta$-unsaturated carbonyl system. The explanation in this case must again lie with the anisotropic effect of the keto group on $C_7$. Since the vinyl proton lies in the plane of the keto group it resonates further downfield than normally expected. This downfield shift of the vinyl resonance is observed in all the deoxy derivatives that lack the epoxy group.

The isopropyl group in citrolin appears to suffer appreciable steric hindrance to free rotation, as each of the methyl bands for this group are doublets with a spacing of about 2 c/s. Lehn[29] has discussed similar isopropyl groups attached to a cycloalkene

---

[29] J. M. Lehn, *Bull. Soc. Chim. Fr.* 1832 (1962).

TABLE 1. NMR SPECTRA OF LIMONIN AND OBACUNONE DERIVATIVES

| Compound | Solvent | α-Furan | β-Furan | Furfurylic $H_{17}$ | $H_{19}$ | $H_1$ | Epoxy $H_{15}$ | C-Methyls | Other assignments | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| Limonin (I) | $CDCl_3$ | 441 (1) | 378 (1) | 327 | 265 (13)<br>285 | 242 | 242 | 78 70 70 65 | | |
| Limonin (I) | $CDCl_3$-DMSO | 447 (1) | 328 (1) | 382 | 270 (13)<br>292 | 247 | 243 | 76 70 67 63 | | |
| Limonin oxime (IX) | $CDCl_3$ | 443 (1) | 380 (1) | 327 | 263 (13)<br>280 | 240 | 229 | 79 73 71 57 | | 30 |
| Limonin diosphenol (VIa) | $CDCl_3$ | 441 (1) | 378 (1) | 324 | 276 | 244 | 245 | 92 89 69 63 | | 4 |
| Limonin diosphenol acetate (VIb) | $CDCl_3$ | 450 (1) | 386 (1) | 330 | 283 | 254 | 245 | 88 88 70 66 | acetate 136 | 4 |
| Deoxylimonin (VIII) | $CDCl_3$ | 449$^b$ | 381$^b$ | 300 | 277 (14)<br>278 | 247 | | 85 76 76 72 | $C_{15}$ vinyl, 410 | 4 |
| Limonyl acetate (Xb) | $CDCl_3$ | 441 (1) | 377 (1) | 332 | 264 | 240 | 208 | 76 73 66 55 | $C_7$-CHOAc, 271 (2)<br>acetate, 127 | 5 |
| Epilimonyl acetate (VIIb) | $CDCl_3$ | 441 (1) | 377 (1) | 330 | 265 (13)<br>270 | 238 | 218 | 76 76 66 62 | $C_7$-CHOAc, 298<br>($J_{a,a}$ 9, $J_{a,e}$ 4·5)<br>acetate, 126 | 5 |
| Limonol (Xa) | $CDCl_3$-DMSO | 443 (1) | 379 (1) | 330 | 265 | 239 | 233 | 75 75 65 50 | $C_7$-CHOH, 206 | 5 |
| Epilimonol (XIa) | $CDCl_3$-DMSO | 446 (1) | 380 (1) | 334 | 267 | 239 | 273 | 74 74 64 56 | $C_7$-CHOH, 224 | 5 |
| Tetrahydrolimonin | $CDCl_3$ | | | 258 (5) | 266 (13)<br>284 | 244 | 228 | 76 72 71 61 | | 37 |
| Methyl hexahydrolimoninate | $CDCl_3$ | | | | 269 | 253 | 227 | 80 80 69 60 | methoxy, 227 | 37 |
| Methyl limonilate (IIb) | $CDCl_3$ | 443 (1) | 376 (1) | 340 | 231 (9)<br>244 | 258<br>(8, 4) | 253 | 100 70 77 77 | methoxy, 219<br>$C_6$ -OCHCO-, 302 | 37 |
| Methyl limonilate (IIb) | $CDCl_3$-DMSO | 458 (1) | 390 (1) | 349 | 234 (8)<br>250 | 267<br>(8, 3) | 256 | 101 81 81 78 | methoxy, 223<br>$C_6$-OCHCO-, 304 | 37 |
| Methyl limonilate oxime (XVI) | $CDCl_3$ | 447 (1) | 381 (1) | 350$^c$ | 225<br>231 (8) | 260<br>(8, 3) | 306 | 101 79 79 79 | methoxy, 222<br>$C_6$-OCHCO-, 346$^c$ | 4 |
| Limonilic acid (IIa) | $CDCl_3$-DMSO | 459 (1) | 392 (1) | 352 | 236 (8)<br>251 | 267<br>(9, 4) | 257 | 100 80 80 78 | $C_6$ -OCHCO-, 306 | 37 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Methyl deoxylimonilate (XVIII) | $CDCl_3$ | 443[b] | 381[b] | 298 | 232 (9)<br>242 | 257<br>(9, 4) | | 103 77 77 77 | methoxy, 220;<br>vinyl, 422<br>$C_6$-OCHCO-, 262 | 4 |
| Methyl deoxylimonate (IVb) | $CDCl_3$ | 444[b] | 383[b] | 311 | 243 (12)<br>252 | 273 (4) | | 109 74 68 63 | methoxy, 222<br>-$CCH_2COO$-, 203 | 4 |
| Citrolin (III) | DMSO | 465[b] | 395[b] | 314 | 292 (12)<br>278 | | | 90 70 65 64 | $C_1C_2$, 412, 377 (10)<br>$C_{15}$ vinyl, 389<br>$C_6$ vinyl, 369 | 37 |
| Limonexic acid (XIX) | $CDCl_3$-DMSO | | | 321 | 271 (13)<br>291 | 249 | 251 | 76 68 68 68 | α-vinyl 361<br>β-vinyl 441<br>anomeric 369, 372 | 5 |
| Isohexahydro limoninic acid (V) | $CDCl_3$ | | | | | | | 81 76 76 76 | | 4 |
| Nomilin (XII) | $CDCl_3$ | 443 (1) | 379 (1) | 325 | | | 227 | 93 87 79 71 65 | $C_1$, 299 ($J_{a,a}$ 6, $J_{a,e}$ 2·5); acetate, 119 | 30 |
| Obacunone (XI) | $CDCl_3$ | 444 (1) | 381 (1) | 327 | | | 219 | 91 91 87 75 67 | $C_1$, 395,<br>$C_2$, 327 (12) | 38 |
| Methyl obacunoate (XIIIb) | $CDCl_3$ | 439 (1) | 378 (1) | 322 | | | 218 | 88 74 72 68 65 | $C_1$, 358,<br>$C_2$ 340 (13); methyl, 216 | 4 |
| Obacunoic acid (XIIIa) | $CDCl_3$-DMSO | 438 (1) | 385 (1) | 328 | | | 219 | 90 75 72 69 67 | $C_1$, 359,<br>$C_2$, 344 (14) | 4 |
| Iso-obacunoic acid (XV) | $CDCl_3$ | 441 (1) | 378 (1) | 331 | | 231 | 262 | 74 74 70 70 68 | | 39 |

---

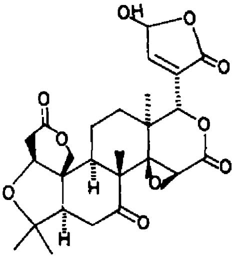
[a] All data at 60 mc. in c/s relative to internal TMS. Coupling constants in parentheses in c/s.

[b] Center of complex multiplet.

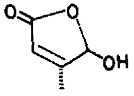
[c] Assignment arbitrary.

ring in the triterpene series in which the two isopropyl methyl groups showed substantial non-equivalence.

In 1947 Emerson[30] reported, as part of an extended study on the bitter principles in citrus, the isolation of small amounts of "substance X," m.p. 315°, from Valencia orange seeds. Kefford and Chandler[31] in Australia later reported the isolation of a compound from citrus seeds, m.p. 315–316°, which they named limonexic acid. There has been no report of a direct comparison, of these two samples but they are most probably identical. Kefford and Chandler also showed that limonin (I), the major bitter principle in citrus, gave limonexic acid[32] when treated with various oxidizing agents. However, the question of whether limonexic acid is an artifact arising during its isolation through air oxidation of limonin, or a true metabolic product in citrus is not settled. In 1957, Arigoni *et al.*[5] reported an improved preparation of limonexic acid from limonin by photosensitized air oxidation under basic conditions. They suggested a reasonable structural relationship of limonexic acid with limonin which indicates that limonexic acid should have structures XIXa or XIXb. The NMR spectrum of a synthetic sample of limonexic acid indicates it is a mixture of the two possible products (XIXa and XIXb) in about equal amounts. The spectrum shows bands at 441, 361, and 369–372 c/s which are assigned to the β-proton in XIXa, the α-proton in XIXb, and the methine proton in both compounds respectively. The resonance for α-protons of β-substituted, α,β-unsaturated butyrolactones comes at about 353 c/s in the cardiac aglycones.[33] α,β-Unsaturated butyrolactone[34] itself shows absorption at 369 c/s for the α-proton and 458 c/s for the β-proton.[35]



XIX a



XIX b

---

[30] O. H. Emerson, *J. Amer. Chem. Soc.* **70**, 545 (1948).

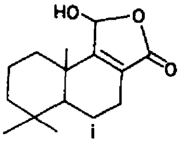
[31] B. V. Chandler and J. F. Kefford, *Australian J. Sci.* **13**, 112 (1951); **16**, 28 (1953).

[32] Synthetic limonexic acid prepared in our laboratories has proved to be tasteless.

[33] R. G. Coombe, T. R. Watson and R. M. Carman, *Chem. & Ind.* 1724 (1962); G. Volpp and Ch. Tamm, *Helv. Chim. Acta* **46**, 219 (1963).

[34] Ref. 8, Spectrum No. 51.

[35] Valdniolide (i) possesses a γ-hydroxy α,β-unsaturated-γ-lactone system[36] which contains a similar methine proton whose chemical shift agrees well with that observed in limonexic acid.



i

[36] H. H. Appel, R. P. M Bond and K. H. Overton, *Tetrahedron* **19**, 635 (1963).

## EXPERIMENTAL[40]

The NMR spectra were taken on a Varian A-60 and are relative to internal TMS. The compounds examined in this study were prepared according to published procedures. The observed physical constants agreed with data previously reported.

---

[37] O. H. Emerson, *J. Amer. Chem. Soc.* **74**, 688 (1952).

[38] O. H. Emerson, *J. Amer. Chem. Soc.* **73**, 2621 (1951).

[39] T. Kamikawa and T. Kubota, *Tetrahedron* **12**, 262 (1961).

[40] Ref. to a company or product does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.