[Chem. Pharm. Bull. 23(1) 125—132 (1975)] UDC 547.92.03 : 581.192

Carbon-13 Nuclear Magnetic Resonance Spectra of Phytoecdysones[1]

Hiroshi Hikino, Toru Okuyama, Chohachi Konno, and Tsunematsu Takemoto

Pharmaceutical Institute, Tohoku University[2]

(Received June 22, 1974)

The natural-abundance ^{13}C nuclear magnetic resonance spectra of certain phytoecdysones and their selected derivatives have been measured at 25 MHz. With the aid of complete noise, off-resonance, and single-frequency proton decoupling techniques, and the shifts which occur on formation of specific derivatives, it has been possible to make assignments of the resonances for the phytoecdysones.

Since the discovery of the first insect moulting hormone, ecdysone, by Butenandt and Karlson,[3] several zooecdysones have been found in arthropods. It has now been well known that congeners of these insect hormones, phytoecdysones, are widely distributed also in the plant kingdom. Indeed some fourty analogs have been isolated from vegetable sources. Undoubtedly the list of the zooecdysones and phytoecdysones will continue to expand.

In elucidation of the structure of the ecdysones, ^1H nuclear magnetic resonance (NMR) spectroscopy, along with mass and optical rotatory dispersion (ORD) spectroscopy, has proven to be highly useful. However, ^1H NMR spectroscopy is limited in usefulness because it often gives spectra too complex to be analyzed well. Although ^{13}C NMR spectroscopy has been expected to be one of the techniques which compensate the short-coming of ^1H NMR spectroscopy, there has been a big obstruction, underdevelopment of the instruments, until quite recently. Since recent development of both proton decoupling and pulsed-Fourier transformation techniques promise wider applications in structural elucidation of natural organic products, systematic examination of the ^{13}C NMR spectra of the phytoecdysones was carried out in the present work in the hope that the carbon resonances are useful for structural determination of any potential congeners. Although analysis of the ^{13}C NMR spectra of the ecdysone relatives has already been performed on ecdysone (**13**) and 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one (**8**) by Lukacs and Bennett[4] and on kaladasterone (**16**) by Canonica, *et al.*,[5] the conclusion drawn in this work is different in some points from the results obtained by the previous authors.[4,5]

Assignments of the carbon atoms in the steroid nuclei of phytoecdysones were first carried out. For this purpose, poststerone (4)[3] was chosen (Table I), because removal of the side-chain system from the phytoecdysones was considered to simplify the spectrum by eliminating six resonances. Partial assignments of the ^1H NMR spectrum of poststerone which are essential for exact proton decoupling techniques, have been reported previously.[6] The signals in the poststerone spectrum occur as three groups, those corresponding to unsaturated carbons at lower field, those corresponding to carbinyl carbons at medium field, and those corresponding to saturated carbons at higher field. Since ketonic carbonyl carbons are known to come into resonance at quite low fields, assignments of C-6 and C-20 to the lowest unsat-

1) This paper forms Part XX in the series on Steroids. Part XIX: K.-Y. Yen, L.-L. Yang, T. Okuyama, H. Hikino, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **22**, 805 (1974).
2) Location: *Aoba-yama, Sendai.*
3) *cf.*, H. Hikino and Y. Hikino, "Fortschritte d. Chem. org. Naturst.," Vol. 28, ed. by W. Herz, H. Grisebach, and A.I. Scott, Springer-Verlag, Wien, 1970, pp. 256—312.
4) G. Lukacs and C.R. Bennett, *Bull. Soc. Chim. France*, **1972**, 3996.
5) L. Canonica, B. Danieli, G. Ferrari, M.A. Haimova, and J. Krepinsky, *Experientia*, **29**, 1062 (1973).
6) H. Hikino, K. Nomoto, and T. Takemoto, *Steroids*, **16**, 393 (1970).

urated resonances at 203.1 and 209.0 ppm are obvious. Between the two resonances, C-6 is assignable to the higher field resonance by comparison of the spectra of the other phytoecdysones (*vide infra*), since the C-20 resonance is unique in the spectrum of poststerone, and the C-6 resonance is common to all the spectra and not likely to be affected by modification of the side-chain. This was corroborated by the finding that the line position of C-20 now allotted to 209.0 ppm is consistent with that (207.3 ppm) of the corresponding resonance in progesterone (**2**). Thus the assignments of the ^{13}C spectra of progesterone in dimethyl sulfoxide and in dioxane have recently been done by Bhacca, *et al.*[7] and those of the spectrum in pyridine were now carried out according to them. The two remaining unsaturated resonances at 164.3 and 122.0 ppm are assigned to C-8 and C-7, respectively, since the former is β to the carbonyl (appearing at lower field) and quaternary while the latter is α to the carbonyl (occurring at higher field) and tertiary, both being consequently identified. Carbons C-2, C-3, and C-14 are hydroxylated in poststerone and appear at lower fields than the remainders. Among the three hydroxylated atoms, C-14 is quaternary and is readily discernible from C-2 and C-3 by off-resonance experiments. The resonances for C-2 and C-3 are not resolved. The off-resonance decoupled spectrum allowed the assignments of the remaining fourteen saturated resonances to three primary (methyl) carbons (17.1, 24.3, and 31.2 ppm), six secondary (methylene) carbons (21.0, 21.9, 30.4, 31.8, 32.2, and 37.8 ppm), three tertiary (methine) carbons (34.4, 51.2, and 59.4 ppm), and two quaternary carbons (38.6 and 48.1 ppm). Among the three methyl carbons, C-21 was assigned to the lowest-field primary atom resonance at 31.2 ppm since it is next to the carbonyl group. Consistency of the chemical shift with that (31.3 ppm) of C-21 of progesterone (**2**) confirms this assignment. Discrimination of C-18 and C-19 would be facilitated by consideration of their chemical shift changes induced by replacements of the C-17 acetyl by an acetoxyl (to **7**), by a cholestane side-chain (to **8**), and by phytoecdysone side-chains (to **10, 11,** and **13**) since alteration of the side-chain would be expected to influence the chemical shift of C-18 more than that of C-19. This argument leads to assignments of the variable resonance at 17.1 ppm to C-18 and of the unaltered resonance at 24.3 ppm to C-19. The next problem is the assignments of the six secondary carbons. The assignments of C-1 and C-4 were made by comparison of the spectra of poststerone, and its 2-acetate (**5**) and 2,3-diacetate (**6**). Thus the C-1 and C-4 resonances must be allotted to the signals at 37.8 and 32.2 ppm, respectively, since they exhibited predictable upfield and downfield shifts (+4.2 and −0.1 ppm, respectively) on going from poststerone to the acetate (**5**). These assignments were further confirmed by the fact that the C-1 and C-4 resonances showed expected downfield and upfield shifts (−0.6 and +3.2 ppm, respectively) on going from the acetate (**5**) to the diacetate (**6**). While the other four methylene resonances did not shift on the acetylation. For disentanglement of the remaining unassigned C-11, C-12, C-15, and C-16 resonances of poststerone, the corresponding resonances of progesterone (**2**) promised to be useful as reference. By application of the hydroxyl substituent parameters of Grant and Roberts,[8] it is expected that introduction of a hydroxyl group at C-14 in progesterone brings about practically no effect on the C-11 resonance, upfield shifts of the C-12 and C-16 resonances, and a downfield shift of the C-15 resonance. This argument leads to the tentative assignments of C-11, C-12, C-15, and C-16 in poststerone to the resonances at 21.0, 31.8, 30.4, and 21.9 ppm, respectively. At this stage, however, definite assignments of the closely appearing resonances for C-11 and C-16, and C-12 and C-15 are highly difficult. The three methines in poststerone, C-5, C-9, and C-17, were readily assignable to the resonances at 51.2, 34.4, and 59.4 ppm, respectively, by single frequency proton decoupling experiments. Of the two unassigned quaternary carbons in poststerone, C-10 and C-13, C-13 was assigned to

7) N.S. Bhacca, D.D. Giannini, W.S. Jankowski, and M.E. Wolff, *J. Amer. Chem. Soc.*, **95**, 8421 (1973).
8) D.M. Grant and E.G. Paul, *J. Amer. Chem. Soc.*, **86**, 2984 (1964); J.D. Roberts, F.J. Weigert, J.I. Kroschwitz, and H.J. Reich, *J. Amer. Chem. Soc.*, **92**, 1338 (1970).

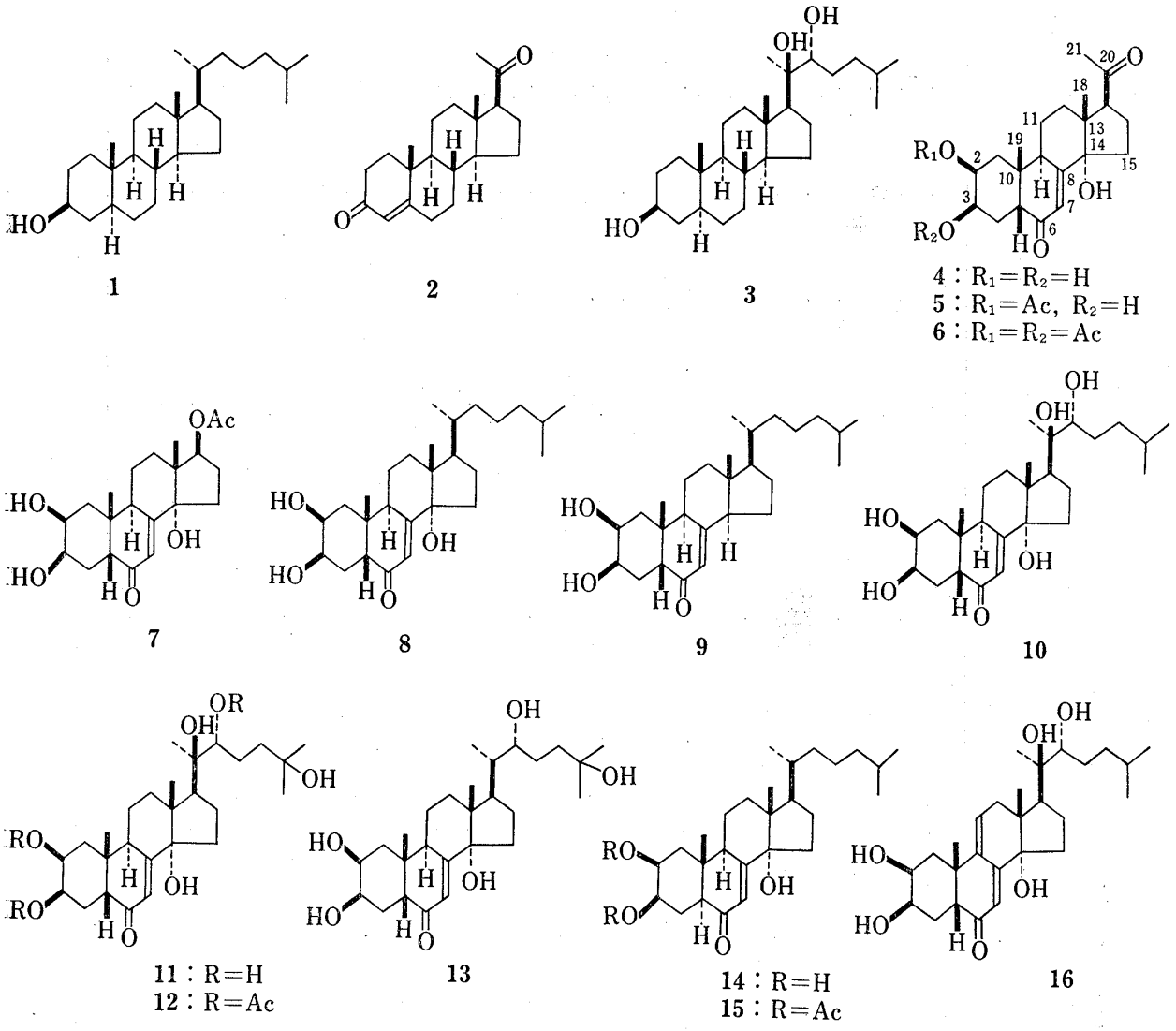

Chart 1

the lower-field resonance at 48.1 ppm because the C-13 resonance in progesterone (**2**) appears at 43.8 ppm and introduction of a hydroxyl at C-14 must give rise a further downfield shift of the C-13 resonance. This assignment was confirmed by the finding that when poststerone was subjected to Baeyer-Villiger oxidation (from **4** to **7**), the C-10 resonance was unchanged but the C-13 resonance suffered from an upfield shift by 0.9 ppm.

Our next endeavor was directed towards the assignments for ponasterone A (**10**),[3] one of the simplest phytoecdysones possessing the C_{27} cholestane skeleton. This was to be facilitated by the use of the two model compounds, 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one (**8**),[9] whose nucleus and side-chain are the same as those of ponasterone A and cholestanol (**1**), respectively, and cholestane-3β,20(*R*),22(*R*)-triol (**3**),[10] whose nucleus and side-chain are to the contrary the same as those of cholestanol and ponasterone A, respectively. Although analysis of the cholestanol spectrum in dioxane in the presence of chloroform has been made by Reich, *et al.*,[11] recent reexamination by Eggert and Djerassi[12] has revealed the revision

9) A. Furlenmeier, A. Fürst, A. Langemann, G. Waldvogel, U. Kerb, P. Hocks, and R. Weichert, *Helv. Chim. Acta*, **49**, 1591 (1966); M.J. Thompson, W.E. Robbins, J.N. Kaplanis, C.F. Cohen, and S.M. Lancaster, *Steroids*, **16**, 85 (1970).
10) H. Hikino, T. Okuyama, S. Arihara, Y. Hikino, T. Takemoto, H. Mori, and K. Shibata, to be published.
11) H.J. Reich, M. Jautelat, M.T. Messe, F.J. Weigert, and J.D. Roberts, *J. Amer. Chem. Soc.*, **91**, 7445(1969).
12) H. Eggert and C. Djerassi, *J. Org. Chem.*, **38**, 3788 (1973).

TABLE I. Carbon-13 Shieldings in Phytoecdysones and Related Substances (ppm from TMS)

Carbon No.	Substance No.														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	37.5	35.8	37.5	37.8	33.6	34.2	37.6	37.9	38.2	37.9	37.8	34.2	37.6	43.4	40.4
2	32.4	34.2	32.3	67.9	72.4	69.1	67.9	68.0	67.7	68.0	68.0	69.2	67.9	69.7	69.1
3	70.6	197.8	70.5	67.9	65.1	67.5	67.9	68.0	67.7	68.0	68.0	67.7	67.9	72.0	72.3
4	39.2	124.0	39.2	32.2	32.3	29.1	32.2	32.4	32.3	32.4	32.2	29.4	32.2	25.0	21.0*[1]
5	45.3	170.1	45.2	51.2	50.9	51.3	51.2	51.3*[1]	50.9	51.3	51.2	51.4	51.2	54.6	53.5
6	29.2	32.7	29.1	203.1	202.2	200.9	203.3	203.4	204.5	203.5	203.4	201.3	203.6	208.1	205.6
7	32.4	32.1	32.3	122.0	122.1	121.8	121.5	121.5	121.5	121.7	121.5	121.2	121.4	122.7	122.5
8	35.8	35.4	35.1	164.3	164.2	164.2	163.9	166.7	165.2	166.0	166.0	166.1	165.5	164.3	164.5
9	54.7	53.7	54.6	34.4	34.2	34.4	34.5	34.5	35.9	34.4	34.3	34.2	34.4	47.5	47.2
10	35.8	38.6	35.7	38.6	38.7	38.5	38.6	38.6	38.2	38.7	38.5	38.4	38.5	22.8	22.9*[1]
11	21.6	21.1	21.5	21.0*[1]	21.0*[1]	20.8*[1]	20.8*[1]	20.9	21.9	21.4	20.4*	20.8	21.0	21.1	21.0
12	40.4	38.6	40.9	31.8*[2]	31.8*[2]	31.8*[2]	28.7*[2]	31.7*[2]	39.0	31.8	31.6	31.7*	31.7*[1]	31.6*	31.6*[2]
13	42.9	43.8	43.7	48.1	48.0	48.0	47.2	47.2	44.7	48.1	48.0	48.0	47.3	46.9	46.9
14	56.7	55.9	56.8	83.9	83.9	83.7	81.5*[3]	84.2	56.2*[1]	84.1	84.1	84.0	83.6	83.9	83.8
15	24.4	23.0	24.4	30.4*[2]	30.4*[2]	30.3*[2]	27.4*[2]	31.4*[2]	22.5*[2]	31.9	31.6	31.4*	31.3*[1]	31.2*	31.1*[2]
16	28.5	24.4	22.5	21.9*[1]	21.8*[1]	21.7*[1]	20.5*[1]	27.3	27.8	21.4	21.3*	22.1	26.5*[2]	27.1	27.2
17	56.7	63.3	55.6	59.4	59.4	59.2	81.7*[3]	51.1*[1]	55.6*[1]	50.0	50.0	50.2	48.1	51.1	51.1
18	12.5	13.3	14.1	17.1	17.1	17.1	16.1	15.8	12.2	17.9	17.6	17.7	15.6	15.7	15.7*[3]
19	12.3	17.1	12.5	24.3	24.0	23.9	24.2	24.3	24.3	24.4	24.3	23.9	24.3	15.7	15.3*[3]
20	36.1	207.3	76.5	209.0	208.8	208.7		36.0*[3]	35.9*[3]	76.7	76.8	76.1	42.3	35.9	35.9
21	19.0	31.3	21.1	31.2	31.2	31.3		19.2	18.7	21.1	21.0	21.1	13.4	19.2	19.3
22	36.5		76.5					36.7*[3]	37.0*[3]	76.7	77.4	80.2	73.8	36.7	36.7
23	24.2		30.1					24.3*[4]	23.9*[2]	30.2	29.8	26.0	25.4*[2]	24.2	24.2
24	39.8		37.1					39.7	39.5	37.1	42.4	41.5	42.7	39.7	39.7
25	28.2		28.2					28.1	27.8	28.1	69.6	69.2	69.6	28.1	28.2
26	22.7		22.5					22.6*[4]	22.5	22.3	27.3	29.2	29.8	22.6	22.2*[1]
27	22.9		23.2					22.8*[4]	22.5	23.3	29.8	30.0	29.8	22.6	22.7*[1]

* The assignments of the asterisked signals are ambiguous and might have to be reversed.

of the assignments for C-12 and C-16. Our identification of ^{13}C resonances of cholestanol in pyridine followed the assignments reported by these authors.[11,12] The chemical shift assignments of the triol (**8**) have been done by Lukacs[4] on the basis of chemical shift considerations comparing with the spectrum of 5β-cholestane whose analysis has been made by Balogh, *et al.*[13] Since comparison of the spectrum of the triol (**8**) assigned by Lukacs[4] and that of poststerone above assigned showed that there are discrepancies between both the assignments for certain carbons, independent analysis of the triol spectrum was performed.

The assignments for C-1 to C-11 and C-19 and those for C-20 to C-27 in the triol (**8**) follow directly from the results for poststerone and cholestanol, respectively. Among the yet unassigned signals, off-resonance decoupling experiments coupled with considerations of the chemical shifts serve to identify the C-13 and C-14 resonances at 47.2 and 84.2 ppm, respectively. The signals at 15.8 and 51.1 ppm for the primary and tertiary carbons C-18 and C-17, respectively, were also identified by off-resonance decoupling. The three signals originating from the C-12, C-15, and C-16 methylene carbons were examined by considerations of substituent parameters. Thus, the effects of a hydroxyl group introduced into C-14 are shielding at C-12 and C-16 and are deshielding at C-15 relative to their positions in cholestanol, leading to the allocation of C-12, C-15, and C-16 to the signals at 31.7, 31.4, and 27.3 ppm, respectively. Since the signals for C-12 and C-15 are separated by only 0.3 ppm, the assignments are arbiturary and the alternative ordering of the signals may be equally valid. As is evident from Table I, modification of the C-17 side-chain brings about essentially no change of the chemical shift of C-12 but causes variation of that of C-16, confirming the validity of the above assignments.

On the other hand, most carbons of the nucleus (C-1-C-12-C-14, C-15, and C-19) and carbons at the end of the side-chain (C-25 –C-27) of the triol (**3**) can be cleanly assigned by comparison with cholestanol. The chemical shifts of the remaining carbons for the triol (**3**) can be estimated as follows. The spectrum of the triol revealed a resonance, other than the C-3 resonance, at low field corresponding to carbons substituted by electronegative atoms. This signal on off-resonance decoupling was separated into a singlet and a doublet and therefore attributed to C-20 and C-22 which coincided in this case. Between the yet unassigned methyl carbons, C-21 was assigned as 21.1 ppm because an lower field shift of the C-21 resonance as compared with the corresponding resonance in cholestanol is anticipated by the occurrence of the effect of the introduced 20,22-glycol moiety. Consequently another primary carbon signal at 14.1 ppm remained for C-18. Allocation of the C-16, C-23, and C-24 to the resonances at 22.5, 30.1, and 37.1 ppm, respectively, were made on the basis of the chemical shift considerations that, due to the introduction of the glycol system at C-20 and C-22, the resonances for C-16 and C-24 are expected to move towards higher field while the resonance for C-23 is predicted to show a downfield shift relative to the corresponding carbons in cholestanol. The only remaining tertiary and quaternary carbons, C-17 and C-13, respectively, were easily pointed out by off-resonance decoupling.

A link in the assignments for the triol (**8**) and the triol (**3**) allowed identification of all the carbons in ponasterone A except for C-13, C-15, C-16, C-17, and C-18. Among these atoms, C-13, C-17, and C-18 are quaternary, tertiary, and primary, respectively, and are easily distinguishable by off-resonance experiments. The displacement values of the C-13, C-17, and C-18 resonances on going between the triol (**8**) and ponasterone A (—0.9, +1.1, and —2.1 ppm, respectively) were found to be identical with those on going between cholestanol and the triol (**3**) (—0.8, +1.1, and —1.6 ppm, respectively). Provided that the chemical shift changes of the C-15 and C-16 resonances on passing from the triol (**8**) to ponasterone A were also as for those on passing from cholestanol to the triol (**3**), the resonances at 31.9 and 21.4 ppm

13) B. Balogh, D.M. Wilson, and A.L. Burlingame, *Nature*, 233, 261 (1971).

in the spectrum of ponasterone A are assigned to C-15 and C-16, respectively, when the parameters to correct for the replacement of the cholestane side-chain by a 20,22-dihydroxycholestane side-chain for the C-15 and C-16 resonances in the latter pair (0 and +6.0 ppm, respectively) were taken into consideration. The noticeable observations are that introduction of a glycol moiety at C-20 and C-22 appear to cause rather upfield shifts (1.1 and 1.1 ppm) of the adjacent carbon (C-17) resonance in the cholestane and the triol (**8**) series. It is now found that, although the line position of the C-23 resonance in ponasterone A is presently settled as 30.2 ppm, a different value (23.2 ppm) has been reported by Canonica[5] for the corresponding resonance in kaladasterone (**16**) whose side-chain is identical with that of ponasterone A. The reason for this discrepancy is so far unknown.

As was discussed above, introduction of a hydroxyl into C-14 in the cholestane cause a number of effects on the neighboring carbons. In order to estimate these effects and, if possible, to obtain more confident evidence for the assignments of the carbon resonances, the shieldings for 2β,3β-dihydroxy-5β-cholest-7-en-6-one (**9**), the 14-deoxy-derivative of the triol (**8**), was analyzed. The resonances in the diol which are displaced from those in the triol are considered to be those for C-9 and C-12 to C-18. Thus, substration of the hydroxyl at C-14 from the triol is expected to cause upfield shifts of the C-13, C-14, and C-15 resonances and down field shifts of the C-9, C-12, C-16, and C-17 resonances. On this basis, consistent assignments can only be achieved if the resonances at 35.9, 39.0, 44.7, 56.2, 22.5, 27.8, and 55.6 ppm in the spectrum of the diol are assigned to C-9, C-12, C-13, C-14, C-15, C-16, and C-17, respectively. The assignments of the C-14 and C-17 signals separated only 0.6 ppm is not without some ambiguity. The shift values for the shielding changes of C-9, C-12, C-13, C-14, C-15, C-16, and C-17 due to the introduction of the C-14 hydroxyl are estimated to be +1.4, +7.3, −2.5, −28.0, −8.9, +0.5, and +4.5 ppm, respectively, the effects on C-13 and C-16 being unexpectedly small. It may be worthy to note that, contrary to the usual γ-effect of hydroxyl groups, the C-14 hydroxyl in the triol (**8**) deshields C-18 by −3.6 ppm relative to the position in the diol (**9**). The displacement of the C-18 resonance on passing from cholestanol (**1**) to the triol (**8**) and from progesterone (**2**) to poststerone (**4**) gave similar values, −3.3 and −3.8 ppm, respectively. The same phenomenon is found also in the effect of another hydroxyl on a methyl situated in the similar relationship; *i.e.*, that of the 5α-hydroxyl on C-19 in the cholestane skeleton.[14]

The assignments for ecdysterone (**11**),[3] the most commonly occurring phytoecdysone, is subsequently to be made. Carbons C-1 to C-22 in ecdysterone are as for ponasterone A and the chemical shifts for these carbons in the latter are essentially matched by resonances in the former. Off-resonance decoupling permits distinction between the quaternary (C-20) and tertiary (C-22) carbinyl carbons which are separated by 0.6 ppm in this case different from those in the spectra of the triol (**3**) and ponasterone A. The remaining carbons (C-23 to C-27) in ecdysterone are identified by usual chemical shift considerations: C-25 is hydroxylated and comes into resonance at much lower field, and consequently C-23 appears at higher field and C-24, C-26, and C-27 at lower field than the corresponding carbons in ponasterone A, leading to the assignments of the spectrum of ecdysterone. Confirmation of the assignments of the C-23 resonance was provided by the acetylation shift (+3.8 ppm) of the signal on passing from ecdysterone to its triacetate (**12**).

The next problem was the analysis of the spectrum of ecdysone (**13**),[3] the first insect moulting hormone and the constituent of certain ferns. For this purpose, ecdysterone was considered to serve as a good reference substance since ecdysone is the 20-deoxy-derivative of ecdysterone and reasonable assignments of the ecdysone spectrum might be performed taking into account the absence of the substituent effect produced by the C-20 hydroxyl.

14) H. Hikino, C. Konno, and T. Takemoto, to be published.

In fact, a comparison of the results for ecdysone and ecdysterone gives an excellent match for the signals of C-1 to C-12, and C-19 as well as C-14, C-15, and C-24 to C-27. Substraction of the hydroxyl from C-20 of ecdysterone must cause a large upfield shift of the C-20 resonance, few ppm shifts towards higher field of the C-17, C-21, and C-22 resonances, and small downfield shifts of the C-13, C-16, and C-23 resonances. In reality, it was found that the resonances of all the carbons in question in the ecdysone spectrum appear at the positions as expected except for the C-13 and C-23 resonances showing upfield shifts. Assignments of the C-23 resonances in both the ecdysone and ecdysterone spectra were corroborated by the acetylation shifts.[4] Comparison of the present assignments for ecdysone with those by Lukacs[4] revealed that the discrepancies between the assignments for C-6 (Δ+27.9 ppm), C-12 (Δ+5.6 ppm), and C-16 (Δ−4.3 ppm) were present as observed in the case of the triol (**8**) spectra. Although the reason for the first discrepancy is not definite, it is considered to be due to a technical mistake in the spectral measurements done by Lukacs. Lukacs carried out the identification of the carbon resonances in the ecdysone spectrum referring to the assignments for the triol (**8**) where the assignments for C-12 and C-16 were reversed, which is the reason for the latter two discrepancies.

The spectrum of the A/B *trans* congener (**14**) relative to the A/B *cis* triol (**8**) was also examined in relation to the spectra of certain phytoecdysones with the A/B *trans* system. Signals predicted to move from the *cis*-triol to the *trans*-triol are those for C-1 to C-10 and C-19. In fact, the results for both triols clearly show the expected similarities for the shieldings of C-11 to C-27. The resonances for C-6 to C-8 were easily identified. In the carbinyl portion of the spectrum, the signals arise from C-2 and C-3 which were distinguished as follows. It has been reported that a carbon bearing an axial hydroxyl resonates at a higher field than a carbon carrying an equatorial hydroxyl. Provided that this finding is still valid in this case and that the effects of the configurational changes at the γ- and δ-positions are negligible, C-2 may be assigned to the higher-field resonance at 69.7 ppm while C-3 assigned to the lower-field one at 72.0 ppm, because on transformation from the *cis*-triol (**8**) to the *trans*-triol (**14**) the orientation of the C-2 hydroxyl is converted to be from equatorial to axial. Off-resonance decoupling permitted distinctions of the primary C-19 (15.7 ppm), the secondary C-1 and C-4 (43.4 and 25.0 ppm), the tertiary C-5 and C-9 (54.6 and 47.5 ppm), and the quaternary C-10 (22.8 ppm). Introduction of the 2β-hydroxyl function into cholestanol is expected to cause several changes, notably at C-1 and C-3 (downfield) and C-4 (upfield), these predictions offering evidence for the assignments for C-1 and C-4 as well as that for C-3. From the spectra of poststerone (**4**) and its diacetate (**6**), it is found that the acetylation shifts of the C-1 and C-4 resonances are +3.6 and +3.1 ppm, respectively. Acetylation of the 2β,3β-glycol system in the triol (**14**) to the diacetate (**15**) brought about the upfield shifts of the C-1 and C-4 resonances by 3.0 and 4.0 ppm, respectively. Based on these observations, it may be concluded that, in 1,2-dihydroxylated systems, a methylene carbon next to a carbon carrying an equatorial hydroxyl shows a larger upfield acetylation shift than a methylene carbon adjacent to a carbon bearing an axial hydroxyl. This conclusion is the opposite to that drawn in monohydroxylated systems.[15] Allocation of the two methine carbons, C-5 and C-9, to the signals at 54.6 and 47.5 ppm, respectively, was performed on the basis of the prediction that acetylation of the C-2 and C-3 hydroxyls (from **14** to **15**) would cause to shift the C-5 signal more than the C-9 signal. The values of the shifts for the C-1—C-10 and C-19 resonances caused by the transformation from the *trans*-isomer to the *cis*-isomer are reported to be +1.1, +0.9, +0.2, +2.0, +6.4, +1.8, +3.7, −0.3, +9.0, +8.2, and −12.3 ppm in the cholestane series[13] while those are now found to be +5.5, +1.7, +4.0, −7.4, +3.3, +4.7, +1.2, −2.4, +13.0, −15.8, and −8.6 ppm in the present triol series. The couple of shift values are not in accord with each other and, in particular, even the sign of the shifts for the C-4 and C-10

15) G.W. Buchanan and J.B. Stothers, *Can. J. Chem.*, **47**, 3605 (1969).

resonances are inverted. This finding demonstrates that the same structural variation in similar series does not necessarily give consistent shifts on the carbon resonances concerned when the environmental features are different.

The results thus obtained will provide the valuable data for the structural analysis of potential phytoecdysones.

Experimental

For measurements of ^{13}C resonances, a JEOL PS-100 NMR spectrometer, equipped with a PFT-100 pulsed-Fourier transform spectrometer and ^{13}C accessories (including an SD-HC hetero-nucleus spin decoupler), was used in combination with a JEC-6 spectrum computer. The operating frequency for ^{13}C resonance was 25 MHz and that for ^{2}H resonance as internal lock 15 MHz. Usually, an 8 mmϕ sample tube was used, but in a case when the amount of sample available for measurement was too small, a 2 mmϕ tube with a 7 mmϕ spherical bulb at its lower end was put concentrically into an 8 mmϕ sample tube. The samples were dissolved in C_5D_5N; approximately 1 or 0.2 ml of 0.5—0.05 M solution was used. Although measurements were usually performed at 28°, poststerone was insufficiently soluble to allow measurements to be carried out, so that higher temperature (50°) was employed. The number of data points used for acquisition of free induction decay in the computer was 8192, and peak positions were computer-calculated on digitized frequency spectra from the sampling rate of free induction decay. Generally from 500 to 50000 scans were averaged but in special cases up to 150000 scans were accumulated. The chemical shifts were expressed in ppm downfield from tetramethyl silane (TMS) as internal reference.

Acknowledgement We wish to thank Dr. J.N. Kaplanis, U.S. Department of Agriculture, for gift of the diol (**9**), and Dr. H. Mori, Teikoku Hormone Mgf. Co., Ltd., for donation of the triol diacetate (**15**). Thanks are also due to Mr. T. Imanari, JEOL Ltd., for measurements of some spectra.