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# SUBSTITUENT EFFECT ON THE METHYL SIGNALS IN THE NMR SPECTRA OF OLEAN-12-EN-3β-OLS EFFECT OF THE HYDROXYL AND ACETOXYL GROUPS

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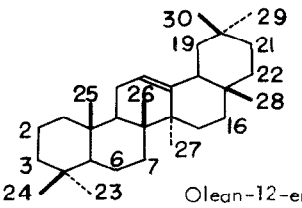
Olean-12-ene, the most common carbon skeleton of the pentacyclic triterpenes, possesses eight methyl groups, or their biogenetic equivalents, on quaternary carbons throughout the frame work. Although the methyl signals in the NMR spectra of compounds of this group have not been used widely for structural studies, their correlation with structure, once established, should be of great use for the study of unknown members which are to be found in nature, because any structural modification should cause systematic changes in the chemical shifts of methyl groups located in the vicinity, as has been found in steroids (1). Tursch et al (2) and Cheung et al (3) attempted to establish these effects on methyl signals but their results are concerned more with the effect of various modifications at 3- and 28-positions and 2-, 3- and 23-positions, respectively. We have examined the changes in the methyl chemical shifts which accompany the introduction of a hydroxyl group at various positions in olean-12-ene and the effect of their acetylation, since hydroxyl group is the most frequently found substituent in natural olean-12-enes and since acetylation is a common procedure in characterization and is also helpful for NMR measurement. The results are tabulated herein together with a few examples of their application.

β-Amyrin (I) was chosen as the parent substance, since the presence of a 3β-hydroxyl group is almost ubiquitous in olean-12-enes and since all the methyl signals have been assigned unequivocally by selective deuteration (4). The compounds examined and their methyl chemical shifts are shown in TABLE I (5). From these values, the effects of hydroxyl and acetoxyl groups on the chemical shifts of each methyl group in the β-amyrin skeleton were calculated, assuming the additivity of each substituent effect. The effect of substituents thus deduced are listed in TABLE II together with the values presented by Tursch (2) and Cheung (3). TABLE II shows that the introduction of a hydroxyl or an acetoxyl group causes a regular and unmistakable change in the chemical shifts of the neighbouring methyl groups but not of the remote methyl groups; e.g. the 23-, 24-, 25- and 26-methyl signals are shifted by the introduction of 6α-hydroxyl or 6α-acetoxyl group, and the 27-, 28-, 29- and 30-methyls are shifted by 22α-substituents. The magnitude of this change

is generally larger for an acetoxyl group than for a hydroxyl group at the same position. Exceptions were observed in three cases: 1) When a methyl group and a substituent are in parallel orientation (1,3-diaxial or 1,3-peridiequatorial) [23-, 27- and 30-methyls with 6α-, 16α- and 22β-substituents, respectively] (6), 2) when an axial methyl group has a substituent at the periequatorial position [27-methyl with 7β-substituents] (7), and 3) when a methyl group and a substituent are in the 1,2-diequatorial relationship [23-, 28- and 29-methyls with 3β-, 22α- and 21β-substituents, respectively].

Applicability of TABLE II is clearly demonstrated for camelliagenin A tetraacetate (XIX) (8) as an example. The calculated chemical shifts in the next page are in good agreement with the observed values (TABLE I).



Olean-12-ene

TABLE I. Methyl Chemical Shifts of Olean-12-enes (5)

| Compd | Substituents | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|
| I | 3β-OH | 0.99 | 0.79 | 0.94 | 0.97 | 1.13 | 0.83 | 0.87 | 0.87 |
| II | 3β-OAc | 0.86 | 0.86 | 0.96 | 0.96 | 1.13 | 0.83 | 0.86 | 0.86 |
| III | 3β, 6α, 28-$(OH)_3$ | 1.31 | 0.98 | 0.98 | 0.98 | 1.19 | $CH_2OH$ | 0.86 | 0.86 |
| IV | 3β, 6α, 28-$(OAc)_3$ | 0.95 | 1.08 | 1.08 | 1.08 | 1.20 | $CH_2OAc$ | 0.88 | 0.88 |
| V | 3β, 28-$(OH)_2$ | 1.00 | 0.79 | 0.95 | 0.95 | 1.18 | $CH_2OH$ | 0.87 | 0.87 |
| VI | 3β, 28-$(OAc)_2$ | 0.87 | 0.87 | 0.96 | 0.96 | 1.17 | $CH_2OAc$ | 0.87 | 0.87 |
| VII | 3β, 7β-$(OH)_2$, 30-$CO_2Me$ | 0.99 | 0.80 | 0.94 | 1.02 | 1.24 | 0.80 | 1.13 | $CO_2Me$ |
| VIII | 3β, 7β-$(OAc)_2$, 30-$CO_2Me$ | 0.87 | 0.89 | 1.00 | 1.09 | 1.21 | 0.79 | 1.13 | $CO_2Me$ |
| IX | 3β, 16α, 28-$(OH)_3$ | 1.00 | 0.79 | 0.92 | 0.92 | 1.35 | $CH_2OH$ | 0.92 | 0.92 |
| X | 3β, 16α, 28-$(OAc)_3$ | 0.86 | 0.86 | 0.97 | 0.92 | 1.28 | $CH_2OAc$ | 0.92 | 0.92 |
| XI | 3β, 16β, 28-$(OH)_3$ | 1.00 | 0.79 | 0.94 | 1.00 | 1.21 | $CH_2OH$ | 0.90 | 0.90 |
| XII | 3β, 16β, 28-$(OAc)_3$ | 0.86 | 0.86 | 0.96 | 1.01 | 1.27 | $CH_2OAc$ | 0.90 | 0.90 |
| XIII | 3β, 22β-$(OH)_2$ | 0.99 | 0.79 | 0.96 | 0.99 | 1.12 | 0.91*1 | 0.88*1 | 1.04 |
| XIV | 3β, 22β-$(OAc)_2$ | 0.87 | 0.87 | 0.98 | 0.98 | 1.15 | 0.98*1 | 0.82*1 | 0.87 |
| XV | 3β, 22α-$(OH)_2$ | 0.98 | 0.79 | 0.93 | 0.98 | 1.15 | 0.98 | 0.93 | 0.93 |
| XVI | 3β, 22α-$(OAc)_2$ | 0.87 | 0.87 | 0.97 | 0.97 | 1.17 | 0.87 | 0.92 | 0.97 |
| XVII | 3β, 23, 28-$(OAc)_3$ | $CH_2OAc$ | 0.84 | 0.99 | 0.96 | 1.15 | $CH_2OAc$ | 0.89 | 0.89 |
| XVIII | 3β, 16α, 21β, 22α, 24, 28-$(OAc)_6$ | 1.03 | $CH_2OAc$ | 0.96 | 0.91 | 1.30 | $CH_2OAc$ | 0.91 | 1.05 |
| XIX | 3β, 16α, 22α, 28-$(OAc)_4$ | 0.86 | 0.86 | 0.97 | 0.92 | 1.31 | $CH_2OAc$ | 0.97 | 1.03 |
| XX | 3β, 16α, 21β, 22α, 28-$(OAc)_5$ | 0.87 | 0.87 | 0.97 | 0.92 | 1.31 | $CH_2OAc$ | 0.92 | 1.05 |
| XXII | 3β, 16α, 28, 30-$(OAc)_4$ (obs.) *2 | 0.86 | 0.86 | 0.97 | 0.93 | 1.28 | $CH_2OAc$ | 0.96 | $CH_2OAc$ |
| | (calc.) | 0.86 | 0.86 | 0.97 | 0.92 | 1.28 | $CH_2OAc$ | 0.92 | 0.92 |

*1 The assignment is ambiguous. *2 See text for the basis of calculation.

TABLE II. Effect of Hydroxyl and Acetoxyl Groups on Methyl Signals (5)*1

| | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|
| Parent (β-amyrin) | 0.99 | 0.79 | 0.94 | 0.97 | 1.13 | 0.83 | 0.87 | 0.87 |
| Acetylation of 3β-OH | -0.13 | 0.07 | 0.02 | -0.01 | 0 | 0 | -0.01 | -0.01 |
| 2α-OH*2 | 0.03 | 0.03 | 0.03 | -0.01 | -0.01 | $CO_2Me$ | -0.02 | -0.02 |
| 2α-OAc*2 | 0.04 | 0.04 | 0.13 | -0.01 | -0.01 | $CO_2Me$ | -0.02 | -0.02 |
| 2β-OH*2 | ? | 0.22 | 0.03 | 0.02 | 0 | $CO_2Me$ | 0.01 | 0.01 |
| 2β-OAc*2 | ? | 0.18 | 0.26 | 0.03 | 0 | $CO_2Me$ | 0 | 0 |
| 6α-OH (III-V) | 0.31 | 0.19 | 0.03 | 0.03 | 0.01 | $CH_2OH$ | -0.01 | -0.01 |
| 6α-OAc (IV-VI) | 0.08 | 0.21 | 0.12 | 0.12 | 0.03 | $CH_2OAc$ | 0.01 | 0.01 |
| 7β-OH (VII-I) | 0 | 0.01 | 0 | 0.05 | 0.11 | (-0.03) | (0.26) | $CO_2Me$ |
| 7β-OAc (VIII-II) | 0.01 | 0.03 | 0.04 | 0.13 | 0.08 | (-0.04) | (0.27) | $CO_2Me$ |
| 16α-OH (IX-V) | 0 | 0 | -0.03 | -0.03 | 0.17 | $CH_2OH$ | 0.05 | 0.05 |
| 16α-OAc (X-VI) | -0.01 | -0.01 | 0.01 | -0.04 | 0.11 | $CH_2OAc$ | 0.05 | 0.05 |
| 16β-OH (XI-V) | 0 | 0 | -0.01 | 0.05 | 0.03 | $CH_2OH$ | 0.03 | 0.03 |
| 16β-OAc (XII-VI) | -0.01 | -0.01 | 0 | 0.05 | 0.10 | $CH_2OAc$ | 0.03 | 0.03 |
| 19α-OH*3 | -0.01 | 0 | -0.01 | 0 | 0.11 | $CO_2Me$ | 0.04 | 0.04 |
| 19β-OH*2 | ? | 0.01 | 0.02 | 0.03 | 0.03 | $CO_2Me$ | 0.01 | 0.01 |
| 21β-OH*3 | -0.01 | 0 | -0.01 | -0.01 | -0.01 | $CO_2Me$ | 0.05*4 | -0.01*4 |
| 21β-OAc*3 | 0 | 0 | 0 | -0.01 | -0.01 | $CO_2Me$ | -0.07 | 0.05 |
| 22α-OH (XV-I) | -0.01 | 0 | -0.01 | 0.01 | 0.02 | 0.15 | 0.06 | 0.06 |
| 22α-OAc (XVI-II) | 0.01 | 0.01 | 0.01 | 0.01 | 0.04 | 0.04 | 0.06 | 0.11 |
| 22β-OH (XIII-I) | 0 | 0 | 0.02 | 0.02 | -0.01 | 0.08 or 0.05 | 0.01 or 0.04 | 0.17 |
| 22β-OAc (XIV-II) | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 | 0.15 or -0.01 | -0.04 or 0.12 | 0.01 |
| 23-OAc (XVII-VI) | —— | -0.03 | 0.03 | 0 | -0.02 | $CH_2OAc$ | 0.02 | 0.02 |
| 24-OAc (XVIII-XX) | 0.16 | —— | -0.01 | -0.01 | -0.01 | $CH_2OAc$ | -0.01 | 0 |
| 28-OH (V-I) | 0.01 | 0 | 0.01 | -0.02 | 0.05 | —— | 0 | 0 |
| 28-OAc (VI-II) | 0.01 | 0.01 | 0 | 0 | 0.04 | —— | 0.01 | 0.01 |
| 29-OH*3 | 0 | -0.01 | 0 | 0.01 | 0 | $CO_2Me$ | —— | 0.03 |
| 29-OAc*3 | 0 | 0 | 0.02 | -0.01 | -0.01 | $CO_2Me$ | —— | 0.07 |
| 30-OAc (XXII-X) | 0 | 0 | 0 | 0.01 | 0 | $CH_2OAc$ | 0.04 | —— |

*1 The down-field shifts are chosen as positive. Values in parentheses are due to the other functional groups.
*2 Taken from ref. 3. *3 Taken from ref. 2. *4 These values are reversed in ref. 2.

| methyl groups | 23 | 24 | 25 | 26 | 27 | 29 | 30 |
|---|---|---|---|---|---|---|---|
| β-amyrin | 0.99 | 0.79 | 0.94 | 0.97 | 1.13 | 0.87 | 0.87 |
| acetylation of 3β-OH | -0.13 | 0.07 | 0.02 | -0.01 | 0.00 | -0.01 | -0.01 |
| 16α-OAc | -0.01 | -0.01 | 0.01 | -0.04 | 0.11 | 0.05 | 0.05 |
| 22α-OAc | 0.01 | 0.01 | 0.01 | 0.01 | 0.04 | 0.06 | 0.11 |
| 28-OAc | 0.01 | 0.01 | 0.00 | 0.00 | 0.04 | 0.01 | 0.01 |
| δ calc. | 0.87 | 0.87 | 0.98 | 0.93 | 1.32 | 0.98 | 1.03 |

Another example is the structural elucidation of cyclamiretin E (XXI), $C_{30}H_{50}O_4$, m.p. 247-249°, $\nu^{KBr}$ 3450 $cm^{-1}$, a new olean-12-ene isolated from the bulbs of Cyclamen europea. XXI afforded a tetraacetate (XXII), m.p. 211-210°. The NMR spectrum of XXII shows six methyl singlets (9), five of which are identical with the calculated values (TABLE I) of the 23- to 27-methyl signals for 3β, 16α, 28-triacetoxyolean-12-ene, suggesting the fourth acetoxyl group be located either at $C_{29}$ or at $C_{30}$. The presence of the hydroxyl group at $C_{30}$ in XXI was confirmed when its identity with dihydrocyclamiretin D (10) was established by a direct comparison.

Caution has, however, to be excercised in applying TABLE II, because, in several cases where hydroxyl groups and/or acetoxyl groups are in 1,2-diequatorial orientation [e.g. acetates of barringtogenol C, theasapogenol A and camelliagenins D and E (11)], the calculated values are somewhat deviated from the observed ones. This may reflect the restricted rotation of the functional groups or the distortion of a ring from the normal chair conformation.

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References and Footnotes

1) Inter al. R.F. Zürcher, Helv.Chim.Acta, 46, 2054 (1963).

2) B. Tursch, R. Savoir, R. Ottinger and G. Chiurdoglu, Tetrahedron Letters, 539 (1967).

3) H.T. Cheung and D.G. Williamson, Tetrahedron, 25, 119 (1969).

4) J. Karliner and C. Djerassi, J.Org.Chem., 31, 1945 (1966), S. Itô, M. Kodama and M. Sunagawa, Tetrahedron Letters, 3989 (1967).

5) All NMR spectra were measured at 60 MHz and/or 100 MHz for $CDCl_3$ solution. The chemical shifts were calibrated against that of $CHCl_3$ (7.27 ppm) and expressed in ppm relative to internal TMS. The largest possible error in chemical shift is 0.01 ppm.

6) This effect is practically the same with that observed by Y. Kawazoe et al, [Chem.Pharm.Bull. (Tokyo), 10, 338 (1962)]. The small down-field shift of the 25-methyl due to the 2β-hydroxyl group (3) is somewhat strange, this could be a typographical error.

7) A similar effect was observed (1) for the 19-methyl signal of steroid with the 11α-substituents.

8) S. Itô, M. Kodama and M. Konoike, Tetrahedron Letters, 591 (1967).

9) Four acetoxyl methyls (δ 2.03, 2.04, 2.06, 2.07) and six carbinyl protons (δ 3.71d, 3.85d, J=12.5; δ 3.93d, 4.08d, J=12.0; δ 4.51t, J=8.5; δ 5.14t, J=4.0) are also observed.

10) D.H.R. Barton, A. Hameed and J.F. McGhie, J.Chem.Soc., 5176 (1962).

11) I. Yosioka, T. Nishimura, A. Matsuda and I. Kitagawa, Tetrahedron Letters, 5973 (1966). S. Itô and T. Ogino, ibid., 1127 (1967).