PART - III

FURTHER STUDIES ON THE STRUCTURE AND STEREOCHEMISTRY OF BARRIGENIC ACID, BARRINIC ACID AND BARRINONIC ACID
Previous work

From the branch wood of Barringtonia acutangula Gaertn the isolation of a new triterpene acid called barrinic acid was reported by Barua et al.\textsuperscript{1,2} and its structure was proposed as $2\alpha, 3\beta, 19\alpha$-trihydroxyolean-12-en-23, 28-dioic acid (XV). They also isolated another new triterpene acid called barrinonic acid from the same source and proposed its structure as $2\alpha, 3\beta$-dihydroxy-19-keto-olean-12-en-23, 28-dioic acid (XVI) (unpublished data\textsuperscript{3}). From the fruits of B. acutangula, Barua et al.\textsuperscript{4} reported the isolation of another acid sapogenin called barrigenic acid and its structure was proposed as $2\alpha, 3\beta, 19\beta$-trihydroxyolean-12-en-23, 28-dioic acid (XVII). Thus barrigenic acid was shown to be the $19\beta$-epimer of barrinic acid. 2,3-diacetyl dimethyl barrigenate and 2,3-diacetyl dimethyl barrinate both gave the same 19-keto compound (XVIII) on oxidation which was again obtained by acetylation of dimethyl barrinonate. Thus Barua et al. isolated both the $19\alpha$ (axial) epimer (barrinic acid) and the $19\beta$-(equatorial) epimer (barrigenic acid) and also the corresponding 19-keto compound (barrinonic acid) from the same plant. This was quite interesting from biogenetic stand point. The evidences putforward by these workers left practically no doubt regarding the nature of the carbon skeleton (i.e. olean-12-ene skeleton) and the locations and configurations
of the oxygen functions at C-2, C-3, C-19 and carboxyl group at C-17 in the above three compounds excepting the configuration of the carboxyl group at C-4 because the above three compounds were not directly converted (and compared by direct comparison of mixed m.p., co-TLC and superimposable IR-spectra with an authentic sample) to a known compound having equatorial carboxyl or hydroxymethylene function at C-4. It is true that triterpenes of the oleanene series having equatorial carboxyl or hydroxymethylene group at C-4 is more commonly encountered in nature than those with corresponding axial epimers. But a number of triterpenes having axial (β) carboxyl or hydroxy methylene group at C-4 have been reported by some workers\textsuperscript{5,6} from plant sources.

Scope of the present work

For \textsuperscript{13}C NMR studies (vide Part II of this thesis) a triterpene of the oleanene series having hydroxyl function at C-19 and C-2 and a carboxyl group at C-4 was necessary to find out the effect of hydroxyl substitution in those two positions and carboxyl group at C-4. As mentioned above Barua et al. isolated previously two such compounds e.g. barrinic acid (XV) and barrigenic acid (XVII) besides barrinonic acid (XVI). The last one has however not been published yet by them.

In view of this the old stock of authentic samples of the above three acids were searched in the laboratory and about 150 mg of a sample designated as dimethyl monoacetyl barrigenate was
obtained. It had m.p. 230-232°. This compound was prepared by Barua et al. (loc. cit.) by saponification of dimethyl triacetyl barrigenate with alkali in cold (Barua et al. - unpublished data). As the above monoacetate was not fully characterized by the earlier workers it became necessary first to characterize the product properly before subjecting it to 13C NMR studies. So this compound was characterized by the present author (details are given later) as 19-acetyl derivative of dimethyl barrigenate (VI). The 13C-NMR spectral study (vide Part II of this thesis) of this compound was found to be quite interesting and moreover it indicated that the configuration of the carbomethoxyl group at C-4 is most probably not equatorial as suggested by Barua et al. but axial. This prompted the present author to study some more derivatives of dimethyl barrigenate with a view to establishing the structure, particularly the stereochemistry of barrigenic acid. This work is described in this part of the thesis.

Characterization of 19-acetyl dimethyl barrigenate

The authentic sample of monoacetyl dimethyl barrigenate was found to be homogeneous in TLC over silica gel G (solvent system: benzene:ethyl acetate = 4:2:8 v/v). It had m.p. 230-32°. It gave blue — violet colour in the Lieberman test. It gave pale yellow colour with tetranitromethane indicating the presence of unsaturation. Its mass spectrum (Fig. 1) did not show the molecular ion peak at m/e 588 (corresponding to the molecular formula C34H52O8 of monoacetyl dimethyl barrigenate) but showed a strong
peak at m/e 528 for the (M-60)$^+$ ion. The mass spectral fragmentation pattern as shown in Scheme 1 clearly located the acetoxy group at C-19. Here the structure of barrigenic acid has been drawn as revised by the present author (i.e. the configuration of the carbomethoxyl group has been shown as axial instead of equatorial).

The mass spectral fragmentation pattern as shown in Scheme 1 leaves no doubt that the monoacetyl dimethyl barrigenate prepared by Barua et al. (loc. cit.) must be the 19-acetyl derivative of dimethyl barrigenate.

The mass spectrum is in complete agreement with the structure (VI, vide page 50, Part II) assigned to the monoacetate. The peak at m/e 320 (ion $a$) arising out of retro Diels-Alder fragmentation involving the 12:13-double bond$^7$ clearly locates the acetoxy group at C-19. Loss of acetic acid from ion $a$ gives rise to the intense peak at m/e 260 for the ion species $b$ which further loses 59 mass unit (-COOCH$_3$) giving rise to the base peak at m/e 201 for the ion species $c$. A very weak peak at m/e 267 was observed for the ion species $d$. Thus it could be safely concluded that the monoacetyl dimethyl barrigenate prepared by Barua et al. is the 19-acetyl derivative.

The above conclusion is further corroborated by the $^1$H NMR spectrum (Fig. 2, CDCl$_3$, 90 MHz) of the above monoacetate. The $^1$H NMR spectrum of the monoacetate (VI) showed six sharp singlets in
Scheme 1

Molecular ion at m/e 588 (not observed)

m/e 528 (strong peak)

-CH₃ → m/e 510
-CH₃COOH → m/e 469

m/e 513

-CH₃COOH

m/e 451

a. m/e 320
b. m/e 260 (very strong peak)
c. m/e 201 (base peak)

H₂CO₃

H₂CO₃

H₂CO₃

H₂CO₃

H₂CO₃
the high field region at $\delta$ 0.73 (3H), 0.80 (3H), 0.88 (3H), 0.94 (3H), 1.12 (3H) and 1.15 (3H) due to the six quaternary methyl groups ($\gamma-\text{CH}_3$). High field shift of one of the methyl groups to $\delta$ 0.73 showed definitely the presence of a 28-carboxethoxy group. The signals for the two carboxethoxy groups appeared as sharp singlets at $\delta$ 3.67 and $\delta$ 3.68. The C-2 axial proton signal appeared as expected as a multiplet centered at $\delta$ 4.06 (the width of this signal indicates the axial orientation of the C-2 proton, $W_2 = 20$ Hz). Unfortunately, the signal for C-3 axial proton overlapped with those for the carboxethoxy groups. The signal for the 19°-axial proton appeared as a clear doublet centered at $\delta$ 4.92 ($J = 10.5$ Hz) which is coupled with the 18β proton, the signal for which appeared as a doublet centered at $\delta$ 2.87 ($J = 10.5$ Hz). The signal for the C-19 acetoxy group (-OCOCH₃) appeared as a sharp singlet at $\delta$ 2.0 (3H). Barua et al.⁴ reported that the signal for the 19-axial (α) - proton appeared as a doublet centred at $\delta$ 3.39 ($J = 11$ Hz) by coupling with 18β-H in the $^1$H-NMR spectrum of 2,3-diacetyl dimethyl barrininate. In the $^1$H-NMR spectrum of 19-acetyl dimethyl barrininate the signal (as observed by the present author) for the 19-axial (α) proton shifted to $\delta$ 4.92 and it appeared as expected as a doublet by coupling with 18β-H. The above data in conjunction with the mass spectral fragmentation pattern of monoacetyl dimethyl barrigenate leaves absolutely no doubt that this compound contains a 19β- (equatorial) acetoxy group. Barua et al.⁴ reported that in the $^1$H NMR spectrum of 2,3-diacetyl-19-hydroxy dimethyl barrigenate the signal for the C-3
axial (α) proton appeared as a doublet centred at δ 4.84 (J=10.5 Hz) by coupling with C-2 axial (β) proton, the signal for which appeared as a broad multiplet centred at δ 5.7 thus indicating the trans diequatorial orientation of the hydroxyl groups at C-2 and C-3 (i.e. 3β OH & 2 α-OH).

Barua et al. commented that the lowest downfield methyl signal in the 1H NMR spectrum of 2,3-diacetyl dimethyl barrigenate appeared at δ 1.27 which was considered to be caused by the presence of a C-23-COOCH₃ group (i.e. equatorial carbomethoxyl group at C-4) and the basis of their argument was the report by Cheung and Williamson that in case of the compound, dimethyl 28, 38-diacetoxyolean-12-en-23, 28-dioate the signal for C-24 methyl appeared at δ 1.38. The present author has carefully gone through the above paper of Cheung and Williamson and found that these authors have themselves cautioned that the downfield shift of C-24 methyl signal supposed to be caused by the presence of 23-COOCH₃ was based on very limited data and so confirmation of this conclusion by studying a number of similar triterpenes is necessary. It may also be pointed out that the compound studied by Cheung and Williamson had a 28-acetoxy group whereas in diacetyl dimethyl barringenate the acetoxy group at C-2 is α.

From the above discussion it appears that Barua and co-workers' explanation of the reason of downfield shift (δ 1.27) of one of the methyl signals is not based on very sound ground.
Preparation of triacetyl dimethyl barrigenate (X)

As the amount of 19-acetyl dimethyl barrigenate (VI) available was not much, the $^{13}$C NMR spectrum (Fig. VI, vide page 55-56, Part II) of this compound was studied first and the material was recovered and the tri-acetate (X) was prepared according to the method of Barua et al. and directly compared with an authentic sample of the triacetate, a very small quantity of which was available in the laboratory. The $^{13}$C-NMR spectrum was also studied. For further comparative study 3,21-diketo ethyl maechaerinate (XI) was also prepared by the present author (vide experimental) by oxidation of ethyl maechaerinate (XIX) with CrO$_3$/pyridine complex. This compound was properly characterized ($M^+$ 496). An authentic sample of ethyl maechaerinate (XIX) was available in the laboratory.

13-C-NMR Spectral Studies

The $^{13}$C NMR studies of 19-acetyldimethyl barrigenate (VI), 2,3,19-triacetyl dimethyl barrigenate (X) and 3,21-diketoethyl maechaerinate (XI) were studied and the assignments of the various carbon resonances of these compounds are shown in Table 2. The assignments were made following the change in chemical shifts produced upon change in oxygenation pattern and using methyl 3ß-hydroxyolean-12-en-28-oate (methyl oleanolate) as a model. For assignments of some particular carbon resonance, comparison has been made with those of some of triterpenoids described in Part II of this thesis.
The assignments of the various carbon signals of 19-acetyl dimethyl barrigenate (VI) has already been described briefly in Part II of this thesis. This spectrum showed six quartets at 23.5, 14.2, 16.3, 24.7, 29.3 and 18.3. Among these at 14.2, 16.3 and 24.7 are assigned to C-25, C-26 and C-27 respectively by comparison with that of methyl oleanolate, (IX) and compounds (V), (II), (III) and (I) as mentioned in Part II of this thesis (vide Table 1 of Part II of the thesis).

The signals for the C-29 (equatorial methyl) and C-30 (axial methyl) methyl carbons in (IX) appeared at 32.8 and 23.4 respectively. Introduction of a 3-OH at C-21 as in methyl machaerinate (V) causes downfield shift of C-20 signal ( 35.7) by 5.3 ppm compared to (IX) and upfield shift of C-29 ( 29.1) and C-30 ( 17.1) by 3.7 ppm and 6.3 ppm respectively. In case of 3, 21-diketo ethyl machaerinate (XI) the 21-keto group causes downfield shift of C-20 ( 45.1) by 14.7 ppm compared to (IX) and upfield shift of C-29 ( 26.2) by 6.6 ppm and downfield shift of C-30 ( 24.4) by 1.0 ppm respectively compared to methyl oleanolate (IX) and compared to methyl machaerinate (V) there is a downfield shift of C-20 by 9.9 ppm and upfield shift of C-9 by 3.1 ppm and downfield shift of C-30 by 6.1 ppm. So the methyl carbon resonance at 29.1 and 17.1 are definitely assigned to C-29 and C-30 respectively in methyl machaerinate (V). The introduction of a 19β-acetoxy group in monoacetyl dimethyl barrigenate (VI) also causes, as in the methyl machaerinate, downfield shift of C-20 ( 35.2),
C-29 (δ 29.3) and C-30 (δ 18.3). Thus the quarternary methyl signals at δ 29.3 and δ 18.3 in monoacetyl dimethyl barrigenate (VI) are assigned to C-29 and C-30 respectively. The remaining methyl signal at δ 23.5 must therefore be due to a methyl group attached to C-4. The C-24 methyl (axial) signal appears at about δ 15.4 - δ 16.0 as in methyl oleanolate, IX, compound IV, in methyl machaerinate (V) etc. So the signal at δ 23.5 cannot be attributed to C-24 (axial methyl) in monoacetyl dimethyl barrigenate i.e. this compound cannot have an axial methyl group (C-24) attached to C-4 which is in contradiction to the structure (dimethyl 2α, 3β, 19β-trihydroxy olean-12-en-23,28-dioate (XX) proposed by Barua et al. (loc. cit.) for dimethyl barrigenate. Therefore the signal at 23.5 (quartet) must be due to the C-23 methyl (i.e. the equatorial methyl attached to C-4). The signal at δ 23.5 has appeared slightly upfield by 4.4 ppm (compared to that of methyl oleanolate (IX) δ 27.9) and 4.7 ppm (compared to that of methyl machaerinate V, δ 28.2). This upfield shift of C-23 methyl signal in 19-acetyl dimethyl barrigenate (VI) can easily be attributed to the presence of a carbomethoxy group at C-24 (i.e. the axial carbomethoxy group attached to C-4) and this finds an excellent parallel in case of dimethyl spergulagenate (II) where the C-29 (equatorial) methyl signal (δ 27.9) has been shifted upfield by 4.9 ppm compared to C-29 signal (δ 32.8) of methyl oleanolate (IX).
Thus the $^{13}$C NMR spectrum of 19-acetyl dimethyl barrigenate (VI) showed that the configuration of the carbomethoxyl group at C-4 should be axial and not equatorial as was originally proposed by Barua et al. It should be mentioned here that the structures (VI) & (X) have been drawn here according to the structure of barrigenic acid as revised by the present author.

The $^{13}$C-NMR spectrum of the triacetyl dimethyl barrigenate (X) has also been studied by the present author and the assignments of the carbon resonances are shown in Table 2.

It is known that acetylation of C-3 equatorial -OH group in olean-12-enes causes slight downfield shift (about 1.2 ppm) of C-24 axial methyl signal (cf. dimethyl spergulagenate II and 3-acetyl dimethyl spergulagenate, III) whereas the resonances of C-23 equatorial methyl remains unaltered (Compare II & III). In case of triacetyl dimethyl barrigenate (X) also the signal at $\delta$ 23.5 remains unaltered compared to 19-acetyl dimethyl barrigenate VI ($\delta$ 23.5) and this is in agreement with the presence of a C-23 equatorial methyl group in barrigenic acid. Thus on the basis of the comparative $^{13}$C NMR studies a revised structure 2α, 3β, 19β-trihydroxy olean-12-en-24, 28-dioic acid (XII) for barrigenic acid is proposed by the present author.

As Barua et al. (loc. cit.) has definitely proged that barrinic acid is the 19α-epimer of barrigenic acid and barrinonic acid is the corresponding 19-keto compound so the structures of
both these compounds should be revised as 2α, 3β, 19α-trihydroxy olean-12-en-24, 28-dioic acid (barrinic acid) (XIII) and 2α, 3β-dihydroxy-19-keto olean-12-en-24, 28-dioic acid (barrinonic acid) (XIV) respectively.

Concluding remarks

The present author wanted to study the 13-c NMR spectra of 2,3-diacetyl dimethyl barrigenate and 2,3-diacetyl-19-keto barrigenate to obtain further evidences in favour of the revised structure but due to paucity of starting material this could not be done. He tried to prepare some 2,3-diacetyl dimethyl barrigenate with the available starting compound with acetic anhydride and pyridine at 0°C but TLC showed the formation of three compounds e.g. triacetyl dimethyl barrigenate, diacetyl dimethyl barrigenate and another compound which was presumed to be a monoacetate as it was more polar in TLC than the diacetate. As the amount of the starting material was small and a mixture of three products were obtained, further attempts to obtain individual acetates by preparative TLC was not made as sufficient amount of material for 13-C-studies was not expected. However, the author wishes to isolate further quantity of the barrigenic, barrinic and barrinonic acid from the plant source in future and carry out further 13-C NMR studies and also some chemical studies on these compounds. The author, however, feels that the proposal of the revised structures are not unjustified on the basis of the data presented here.