

STUDIES ON INDIAN MEDICINAL PLANTS—XXXIX¹

REINVESTIGATION OF THE LACTONES AND BROMO DERIVATIVE OF BETULINIC ACID

B. ACHARI and S. C. PAKRASHI*

Indian Institute of Experimental Medicine, Calcutta-700032, India

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Abstract—Isobornyloxy aluminium dichloride reduction of the ketolactone **2a** of betulinic acid (**1a**) gave the axial alcohol **2b** as the preponderant product. Hydroxylactone B of **1a** therefore must have the equatorial OH function contrary to our previous conclusion. The so-called "hydroxylactone A" has been found to be the ring A rearranged product **3a**. Reexamination of earlier work has shown that **1a** yields a mixture of **2e** and **5a** in presence of HBr-AcOH, and a mixture of **2d** and **3a** with refluxing formic acid. Bromination of **1a** affords **1e**.

We reported² that treatment of betulinic acid (**1a**) with glacial acetic acid: conc sulphuric acid: water (50:1:1) afforded a high melting ($> 330^\circ$) acetyl lactone which on saponification yielded hydroxylactone B, m.p. $> 330^\circ$, $[\alpha]_D + 57^\circ$. The latter was earlier obtained on hydrolysis of the formyl lactone of **1a** by Robertson *et al.*³ who also reported hydroxylactone A, m.p. $> 320^\circ$, $[\alpha]_D + 75.2^\circ$, prepared from **1a** or its acetate (**1b**) with hydrogen bromide in acetic acid or by refluxing **1b** with formic acid, followed by saponification in each case. Chromic acid oxidation of both the hydroxylactones A and B to the same ketolactone and other considerations led Davy *et al.*⁴ to believe the two hydroxylactones to be identical. We, however, observed that the product prepared through formic acid treatment of **1b** could be easily differentiated from hydroxylactone B by TLC and IR and as such accepted the suggested⁵ epimeric nature of the two compounds at C-3 centre.

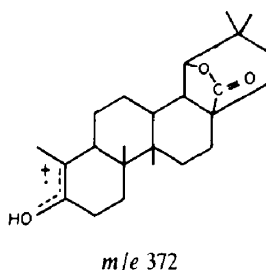
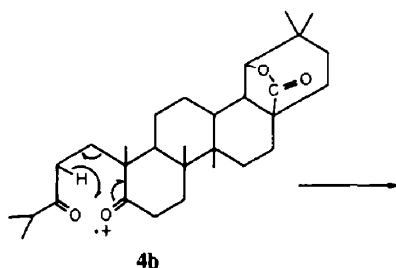
Now, the ketolactone from hydroxylactone B predominantly regenerated the original alcohol on reduction with sodium borohydride or sodium-alcohol.^{3,6} On the other hand, the preparation of hydroxylactone A from **1b** was not expected to involve any inversion at the C-3 centre. These observations could be explained either by assuming α (axial) configuration for the OH group in hydroxylactone A and consequently in **1a** or by presuming the above reductions of the ketolactone to take an abnormal course to yield axial alcohol (in hydroxylactone B) as the major product. Since there could be no doubt about the 3β configuration of the alcoholic function in **1a**, we were forced⁷ to envisage a conformational transmission effect originating in ring E of the ketolactone to rationalise what appeared to be an unusual reduction.

In a recent investigation on the reduction of 3-ketotriterpenoids¹ with isobornyloxy aluminium dichloride—a stereospecific reagent⁸ known to yield preponderantly the axial alcohol—we obtained from the ketolactone (**2a**) a new product, m.p. 306° , $[\alpha]_D + 45^\circ$, designated as epibetulinic acid lactone (**2b**) different from both hydroxylactones A and B. It was also found to be the major product of Meerwein-Pondorff-Verley reduction

of the ketolactone, the other product being hydroxylactone B. The NMR spectra, besides showing a one proton singlet around δ 4.0 ppm for α -proton on the lactone ring common to the three compounds, revealed that the new compound contains a 3α (axial) alcoholic function (1H, t, $W_{1/2} = 5$ Hz, δ 3.39) which must be equatorial (1H, q, $W_{1/2} = 16$ Hz, δ 3.25) in hydroxylactone B(**2c**) as would be expected of normal reductions, while the so-called "hydroxylactone A" was not an alcohol at all. These observations therefore rendered the very basis of our previous conclusions⁷ invalid and prompted us to undertake the thorough reexamination of the reactions of formic acid, hydrogen bromide-acetic acid and of bromine on betulinic acid or its acetate^{3,4} to elucidate the structure of "hydroxylactone A" and to remove confusion in the literature.

Action of formic acid. Betulinic acid (**1a**) on refluxing with 98% formic acid afforded a mixture of formyl lactone B (3β - formyloxy - 18α - oleanano - 28,19 β - lactone; **2d**)^{3,4} and the so-called "hydroxy-lactone A".⁷ The latter, along with acetyl lactone B (3β - acetoxy - 18α - oleanano - 28,19 β - lactone; **2e**)^{3,4} was also obtained from **1b** on similar treatment. The same "hydroxylactone A" was also obtained⁷ by us on treating **1a** with PCl₅.

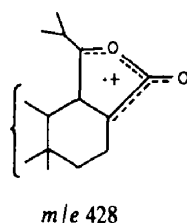
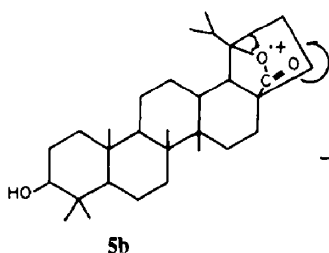
"Hydroxylactone A" analysed for C₃₀H₄₆O₂ (M⁺ 438). The IR and NMR spectra supported the presence of a lactone ring and evidently there cannot be any other oxygen function in the compound. Its mass spectrum, unlike that of hydroxylactone B, showed the M-43 ion as the base peak. Formation of the compound from **1a** involving loss of a molecule of water would, on mechanistic ground, require ring A contraction with better stabilisation of the double bond at 3(5) under acidic condition leading to structure **3a**. This was confirmed by its oxidation with osmium tetroxide. Under the condition α -apoallobetulin (**3b**) was cleaved to the diketone (**4a**).⁹ "Hydroxylactone A" afforded the expected diketolactone (**4b**). The mass spectrum of the product (M⁺ 470) showed M-43 and M-71 fragments besides the base peak at *m/e* 372 which could arise by McLafferty rearrangement as follows:



The structure of "hydroxylactone A" could thus be established as A - neo - 18 α - olean - 3(5) - eno - 28,19 β - lactone (3a). It was earlier reported¹⁰ from montan wax.

Compound 3a was also the predominant product when 2d was refluxed with excess of formic acid, while heating 1b with the same acid at 100° yielded mainly 2e. Evidently, refluxing is a necessary condition for ring A contraction with the reagent. Though PCl₅, P₂O₅ or Fuller's earth are known to bring about such change, this appears to be the first report of ring contraction in triterpene series with formic acid. Incidentally, the present work indicates that β -apoallobetulin, a minor product (< 2% yield) obtained by Ruzicka *et al.*¹¹ on refluxing betulin (1c) with the same reagent, was possibly an impure variety of α -apoallobetulin.⁹

Action of HBr-acetic acid. On treatment with this reagent, 1a or 1b yielded two major products, one of which was identified as 2e. The other component showed the IR peaks for acetoxy and γ -lactone functions similar to 2e; the NMR spectrum confirmed the presence of an equatorial acetoxy function at C-3 but did not exhibit the singlet near δ 4.0 ppm for the α -proton on the lactone ring. Structure 5a (β - acetoxyilupano - 28,19 β - lactone) thus appeared likely for the compound. This conclusion was supported by the mass spectrum of the corresponding hydroxylactone (5b) which was differentiated from that of 2c by the base peak at M-28 most probably due to the loss of an ethylene rather than carbon monoxide molecule as shown below:



The structural assignment was finally confirmed by direct comparison[†] of the hydroxylactone with an authentic specimen prepared earlier by Allison *et al.*¹² from 1a by mercuric acetate oxidation followed by hydrogenation.

Action of bromine. Robertson *et al.*³ reported a monobromolactone by adding bromine in acetic acid to an ethereal solution of 1b. Later, Bruckner Jr. *et al.*¹³ obtained monobromoacids by treating 1a or 1b with bromine in chloroform. We have now observed that even Robertson's procedure leads to monobromoacids rather than the lactones.

The product from 1b analysed for C₃₂H₄₉O₄Br (M⁺ 576, 578) and formed a methyl ester. Structure 1d has been assigned to the bromoacid on the basis of following evidences: the mass spectrum of 1d showed a peak at *m/e* 395 which did not contain bromine and could conceivably arise from the M-60 ion by expulsion of the side chain with two hydrogen transfers. The NMR spectrum exhibited, *inter alia*, a slightly broad singlet (2H) at δ 3.97 ppm for the -CH₂Br protons and two singlets (1H each) at δ 5.02 and 5.15 ppm for the =CH₂ protons.

The bromination of betulinic acid thus takes a course similar to that of lupeol reported by Gupta *et al.*¹⁴ who also observed the isomerisation of the allyl to the vinyl bromide on keeping with hydrobromic acid for a longer period. The spectral data of our compounds however excludes any such possibility under the conditions used.

The results of lactonisation and bromination reactions of betulinic acid reported so far by earlier workers and those now observed by us are summarised in Table 1. It may be noted that different possible products involving the double bond with or without rearrangement of rings A and E could be characterised. The difficulty in purifying the resulting complex mixture explains the confusion prevailing in the literature which could be clarified in the present work.

EXPERIMENTAL

All m.p.s were determined in open capillaries and are uncorrected. Optical rotations were measured in CHCl₃ in a Hilger-

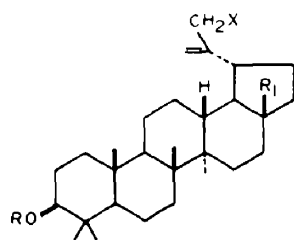
Watts M-511 Microptic photoelectric polarimetre and IR spectra as nujol mulls (unless otherwise specified) with a Perkin-Elmer Infra-cord (Model 137) instrument. Mass spectra were recorded in RMU-6L spectrometer at 80 eV using the direct inlet system.

Petroleum ether refers to fraction of b.p. 60-80°. TLC was done on silica gel G plates using (A) C₆H₆, or (B) C₆H₆: EtOAc (9:1) as solvent system. Anhydrous Na₂SO₄ was used as the drying agent and silica gel (BDH) as adsorbent for chromatography.

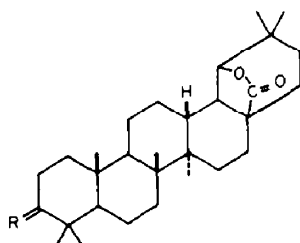
Isobornyloxy aluminium dichloride reduction of 2a

Isolation of lactone 2b and its acetate 2f. Reduction of 2a (0.17 g) by this reagent in the usual way¹ furnished 2c and its 3-epimer (2b) in 17:83 ratio. Crystallisation of 2b from CHCl₃-pet. ether gave colourless prisms, m.p. 304-306°, [α]_D +45.0° (c 0.60); ν_{\max} 3480 (OH), 1760 (C=O), 1155, 1120, 1070, 995, 970, 930 cm⁻¹. (Found: C, 78.59; H, 10.46. C₃₀H₄₄O₃ requires: C, 78.89; H, 10.59%).

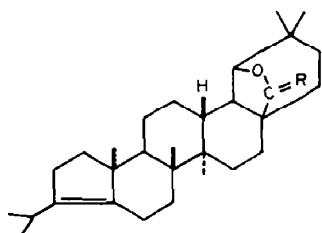
[†]We are grateful to Dr. W. Lawrie of the University of Strathclyde for the comparison.



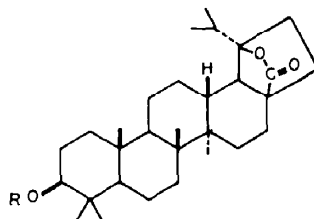
- 1a: R = X = H, R₁ = COOH
 b: R = Ac, R₁ = COOH, X = H
 c: R = X = H, R₁ = CH₂OH
 d: R = Ac, R₁ = COOH, X = Br
 e: R = H, R₁ = COOH, X = Br
 f: R = Ac, R₁ = COOMe, X = Br
 g: R = H, R₁ = COOMe, X = Br



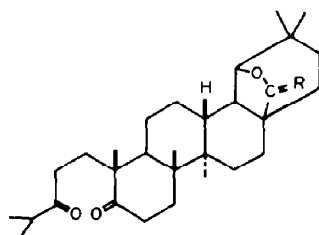
- 2a: R = O
 b: R = βH, αOH
 c: R = αH, βOH
 d: R = αH, βOCHO
 e: R = αH, βOAc
 f: R = βH, αOAc



- 3a: R = O
 b: R = H₂



- 5a: R = Ac
 b: R = H



- 4a: R = H₂
 b: R = O

Table 1. Summary of products obtained from 1a or 1b

Compound	Reagent	Condition	Product(s)	
			Reported	Present work
<u>1a</u> or <u>1b</u>	HBr-AcOH	room temp.	acetyl lactone A (<u>2a</u>) ^{3,4}	<u>2a</u> + <u>3a</u>
<u>1b</u>	HNO ₂ H	reflux	acetyl lactone A (<u>2a</u>) ^{3,4}	<u>2a</u> + <u>3a</u>
		100°	-	<u>2a</u>
<u>1a</u>		reflux	<u>2a</u> ^{3,4}	<u>2a</u> + <u>3a</u>
<u>1b</u>	Br ₂	room temp. ²	monobromolactone ³ m.p. 290°d.	<u>1a</u>
		cold ²	monobromolactone ¹³ m.p. 280°d.	-
<u>1a</u>		cold ²	monobromolactone ¹³ m.p. 250-251°d.	-
		room temp. ²	-	<u>1a</u>

²in ether-AcOH³in CHCl₃

On heating with Ac_2O -pyridine at 100° for 3 hr **2b** formed the acetate **2f**, crystallised from MeOH as needles, m.p. $234\text{--}236^\circ$, $[\alpha]_D + 17.6^\circ$ (c 1.02); ν_{max} 1770 (lactone C=O), 1730, 1250 ($-\text{OCOCH}_3$), 1120, 1020, 980, 945, 930, 910 cm^{-1} .

Meerwein-Ponndorf-Verley reduction of 2a. Al-foil (25 mg) was dissolved in dry isopropanol (5 ml), containing a crystal of HgCl_2 and a drop of CCl_4 , by refluxing for 2 hr; **2a** (90 mg) was added and refluxing continued for 4 hr more, using an air condenser. The mixture was left overnight, acidified with cold 4N H_2SO_4 , extracted with CHCl_3 . The crude product (90 mg) could be resolved by rechromatography into **2b** (52 mg) and **2e** (25 mg).

Action of HCO_2H on betulinic acid (**1a**) or its acetate (**1b**)

Isolation of lactone 3a. A mixture of **1a** (0.5 g) and 98% HCO_2H (30 ml) was refluxed for 5 hr, cooled, diluted with water and extracted with CHCl_3 , the combined organic layer was washed with water till free from acid, dried and distilled. The crude product (0.45 g) yielded **2d** on crystallisation from CHCl_3 -MeOH as needles (0.12 g), m.p. $> 320^\circ$, $[\alpha]_D + 54.3^\circ$ (c 0.46); ν_{max} 1765 (lactone C=O), 1705, 1175, 970, 950, 925 cm^{-1} . Chromatography of the mother liquor recovered a further amount (30 mg) of **2d** (total yield 28%) with 40% C_6H_6 in pet. ether while elution with 5–10% C_6H_6 in pet. ether yielded **3a** (0.25 g, 48%) crystallised from C_6H_6 in fine needles, m.p. $294\text{--}296^\circ$ d, $[\alpha]_D + 79.7^\circ$ (c 0.69) (Lit.¹⁰ m.p. $292\text{--}294^\circ$, $[\alpha]_D + 79.2^\circ$; ν_{max} 1760 (lactone C=O), 970, 950, 940, 920 cm^{-1} ; m/e 438 (M^+ , 13%), 423 (15), 395 (100), 135 (20), 121 (24). (Found: C, 82.39; H, 10.88. $\text{C}_{10}\text{H}_{14}\text{O}_2$ requires: C, 82.13; H, 10.57%).

Refluxing **1b** (0.5 g) with 98% HCO_2H (30 ml) for 6 hr yielded, after similar work-up, 0.45 g of a crude product. Crystallisation from C_6H_6 afforded 0.2 g of **3a** while chromatography of the mother liquor afforded an additional 85 mg of it (total yield 65%) in fractions eluted with 5–10% C_6H_6 in pet. ether. Later fractions gave 60 mg (12%) of **2e**.

In another experiment **1b** (0.5 g) was heated with 98% HCO_2H (30 ml) at 100° for 6 hr. Usual work-up followed by crystallisation from CHCl_3 -MeOH yielded **2e** (0.385 g; 77%).

Osmium tetroxide oxidation of 3a

Isolation of the diketolactone 4b. To a soln of **3a** (0.1 g) in pyridine (5 ml) was added OsO_4 (60 mg) and the soln was stirred at room temp. for 2 hr. It was left overnight, treated with a soln of NaHSO_3 (0.2 g), stirred for 15 min at room temp., extracted with CHCl_3 , organic layer washed successively with 2N HCl , NaHCO_3 aq and water, dried and distilled. The crude product was extracted with hot C_6H_6 to obtain a coloured material containing a major component (TLC). Chromatography yielded the major product (0.115 g) in 10–20% CHCl_3 in C_6H_6 eluents. Crystallisation from MeOH furnished **4b** (65 mg) appreciably soluble in most organic solvents except pet. ether. Recrystallisation from rectified spirit afforded flakes, m.p. $166\text{--}167^\circ$, $[\alpha]_D + 32.7^\circ$ (c 0.52) (Lit.¹⁰ m.p. $167.5\text{--}169.5^\circ$, $[\alpha]_D + 26.5^\circ$); m/e 470 (M^+ , 3%), 455 (16), 452 (28), 427 (35), 409 (19), 399 (12), 391 (5), 381 (26), 372 (100), 328 (17), 235 (30), 189 (58), 126 (72), 107 (75).

Action of HBr -AcOH on **1a and **1b**.** A soln of **1a** (0.5 g) in glacial AcOH (40 ml) was saturated at room temp. with dry HBr gas¹¹. The soln was left for 4 days and then diluted with water and extracted with CHCl_3 . The crude product (0.6 g) obtained from the organic layer by the usual work up was crystallised from CHCl_3 -EtOH and then from C_6H_6 to yield 0.11 g (20%) of **2e**, m.p. $> 320^\circ$, $[\alpha]_D + 58.0^\circ$ (c 0.81); ν_{max} 1760 (lactone C=O), 1730, 1250 ($-\text{OCOCH}_3$) cm^{-1} .

Chromatography of the mother liquor yielded on elution with 20–40% C_6H_6 in pet. ether, another solid (0.26 g), which recrystallised from MeOH to afford **5a** (0.12 g; 22%), m.p. 282° d, $[\alpha]_D + 46.7^\circ$ (c 0.60) (Lit.¹² m.p. $299\text{--}300^\circ$, $[\alpha]_D + 49^\circ$; ν_{max} 1770, 1740, 1250, 1025, 980, 970, 905 cm^{-1}). It was saponified with 2% KOH in EtOH, benzoylated ($\text{C}_6\text{H}_5\text{COCl}$ -pyr. at 100° for 3 hr) and the crude product purified by repeated crystallisation from CHCl_3 -MeOH. Alkaline hydrolysis of the benzoate and fractional crystallisation of the product from MeCN furnished **5b**, m.p. 319° , $[\alpha]_D + 38.0^\circ$ (c 1.00) (Lit.¹² m.p. $314\text{--}316^\circ$, $[\alpha]_D + 41^\circ$; ν_{max} 1760, 1170, 1030, 905, 825, 775 cm^{-1} ; $\nu_{\text{max}}^{\text{CHCl}_3}$ 3400, 1760 cm^{-1} ; m/e 456 (M^+ , 15%), 438

(95), 428 (100), 423 (25), 410 (5), 395 (28), 369 (25), 356 (12), 329 (13), 261 (11), 248 (12), 234 (12), 220 (60), 207 (54), 206 (75), 203 (46), 202 (52), 189 (92), 187 (40), 135 (66), 121 (64), 107 (63).

Under similar condition **1b** (0.5 g) yielded **2e** (0.11 g; 22%) and **5a** (0.12 g; 24%).

Bromination of **1a** and **1b**

Preparations of 1d–1g. To a soln of **1b** (0.25 g) in ether (5 ml) was added 2.5 ml of a 5% soln of Br_2 in AcOH dropwise at room temp. The soln was allowed to stand for 3 hr when a white solid separated. It was filtered and washed with MeOH. The combined mother liquor was diluted with water, extracted with ether, organic layer washed with aq. NaHSO_3 followed by NaHCO_3 aq and water, dried and distilled. Since TLC showed the precipitated solid and the extracted residue to be the same, they were combined (0.28 g) and crystallised from CHCl_3 -MeOH to obtain **1d** in needles, m.p. 280° d. (Lit.¹³ m.p. 280° d.), $[\alpha]_D - 20.0^\circ$ (c 0.59); ν_{max} 3300–2600 (br), 1735, 1700, 1250, 1030, 980, 905 cm^{-1} ; mass spectrum: m/e 578, 576 (M^+ , 1%), 518, 516 (4), 503, 501 (3), 498 (6), 497 (6), 475, 473 (4), 452 (4), 451 (4), 438 (25), 437 (24), 423 (6), 395 (8), 249 (11), 248 (11), 233 (43), 205 (26), 189 (100), 187 (62), 175 (21), 161 (23), 135 (43), 121 (43), 107 (42). (Found: C, 66.14; H, 8.58. $\text{C}_{12}\text{H}_{16}\text{BrO}_4$ requires: C, 66.51; H, 8.55%).

A methanolic soln of **1d** (30 mg) was treated with excess of ethereal CH_3N_2 soln in cold for 1 hr, solvent removed and the product (30 mg) crystallised from MeOH to yield **1f** as silky needles, m.p. $221\text{--}222^\circ$, $[\alpha]_D - 2.2^\circ$ (c 0.91); $\nu_{\text{max}}^{\text{CHCl}_3}$ 1725 cm^{-1} .

Betulinic acid (**1a**, 0.23 g) was partially dissolved in CHCl_3 (ca 10 ml) and a 5% soln of Br_2 in CHCl_3 (ca 2 ml) was dropwise added to it until the yellow colour persisted on stirring. It was left at room temp. for 1 hr, extracted with CHCl_3 , washed once with NaHSO_3 aq to remove colour, then with water till acid-free, dried and distilled. The residue was crystallised from CHCl_3 -MeOH to obtain **1e** as fine needles (95 mg), m.p. $255\text{--}256^\circ$ d. (Lit.¹³ m.p. $250\text{--}251^\circ$ d.), $[\alpha]_D + 4.7^\circ$ (c 0.85, EtOH); ν_{max} 3300, 3100–2500 (br), 1700, 1190, 1030, 985, 907, 760, 720 cm^{-1} . With CH_3N_2 it formed a methyl ester (**1g**), crystallised from MeOH as needles, m.p. $139\text{--}143^\circ$ d. (Lit.¹³ m.p. $138\text{--}140^\circ$ d+), $[\alpha]_D - 20.8^\circ$ (c 1.06); ν_{max} 3250, 1730, 1190, 1150, 1130, 1050, 1030, 985, 910 and 720 cm^{-1} ; m/e 550, 548 (M^+ , 2%), 469 (43), 451 (16), 409 (25), 391 (6), 262 (18), 247 (35), 207 (78), 189 (100), 175 (30), 135 (61), 119 (53), 107 (50).

The residue from the mother liquor of **1e**, when treated with excess of ethereal CH_3N_2 , yielded a further 0.105 g of **1g**.

Acknowledgements—The authors are grateful to Prof. H. N. Khastgir, North Bengal University, India for a generous gift of betulinic acid, and to Dr. U. R. Ghatak, Indian Association for the Cultivation of Science, Calcutta, for the NMR spectra.

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[†]Ascribed, possibly by mistake, to **1f** in the original work¹³.