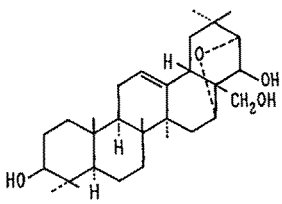
keto ester ($C_{31}H_{44}O_5$, m.p. 217–218°, Anal. calc. for $C_{31}H_{44}O_5$: C, 74.96; H, 8.93; found: C, 74.46; H, 9.12) and methyl hydroxy keto ester ($C_{31}H_{46}O_5$, m.p. 274–277°, $[\alpha]_D$ + 77.5° ($CHCl_3$); Anal. calc. for $_{31}H_{46}O_5$: C, 74.65; H, 9.29; found: C, 74.06; H, 9.33). The formation of the above two esters indicated the presence of one primary hydroxyl group in barringtogenol D. The second ester formed an orange-red mono-2:4-dinitrophenyl hydrazone ($C_{37}H_{50}O_8N_4$, m.p. 258°; Anal. calc. for $C_{37}H_{50}O_8N_4$: C, 65.49; H, 7.37; N, 8.26; found: C, 65.34; H, 7.46; N, 8.28). A band at 1765 cm^{-1} in the infra-red spectrum of the methyl diketo ester suggested the presence of a five-membered ring ketone involving the oxide function[6]. This behaviour of barringtogenol D toward chromium trioxide oxidation finds a remarkable parallel in the case of aescigenin[6] and offers considerable support for similar disposition of the groups in barringtogenol D.

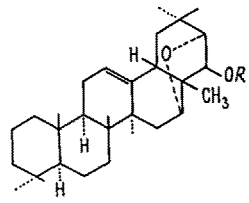
After considering all of the available positions for the site of the 1:3 glycol in a β-amyrin nucleus, and by the process of elimination in the light of various reactions, it is suggested that barringtogenol D has this group at 28:22β-position.

Regarding the oxide linkage, no evidence could be obtained for its location in barringtogenol D. Because of the similarity in some behaviour of aescigenin[6] and the formation of the methyl diketo ester, involving the oxide linkage and C_{22}-ketone, it is considered likely that barringtogenol D also has its oxide bridge linked between 16α- and 21α-position. Thus, a 3β:22β:28-trihydroxy-16α:21α-oxido-olean-12-ene structure (I) is tentatively suggested for barringtogenol D. The confirmation of this structure was accomplished as follows:

Barringtogenol D was oxidized with chromium trioxide-acetic acid in benzene and the neutral oxidation product, on HUANG-MINLON variation of Wolff-Kishner reduction[11], gave a desoxy compound ($C_{30}H_{48}O_2$, m.p. 198–200°, $[\alpha]_D$ + 61° ($CHCl_3$); IR-band at 3400 cm^{-1} and 1100 cm^{-1}; Anal. calc. for $C_{30}H_{48}O_2$; C. 81.77; H, 10.92; found: C, 81.67; H, 10.71). The above compound furnished an acetate ($C_{32}H_{50}O_3$, m.p. 201–203°, $[\alpha]_D$ + 76° ($CHCl_3$); Anal. calc. for $C_{32}H_{50}O_3$: C, 79.61; H, 10.44; found: C, 79.45; H, 10.41). The identity of the desoxy compound and its acetate with 22β-hydroxy-16α:21α-oxido-olean-12-ene (IIa) and its acetate (IIb) respectively, prepared from aescigenin[6], was established by comparison of their physical constants and also by mixed melting point determinations with corresponding authentic samples kindly supplied by Dr. ARIGONI of Zürich (Switzerland). The structure and stereochemistry of barringtogenol D may be represented as (I)[12].



I



IIa, R=H; b, R=OCCH$_3$

Zusammenfassung. Die Struktur des aus der Frucht von *Barringtonia acutangula* Gaertn. isolierten neuen Triterpenoiden, Barringtogenol D, ist als 3β:22βoxy-16α:22α-Oxido-olean-12-en ermittelt worden.

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Department of Chemistry, Bose Institute, Calcutta (India), November 10, 1961.

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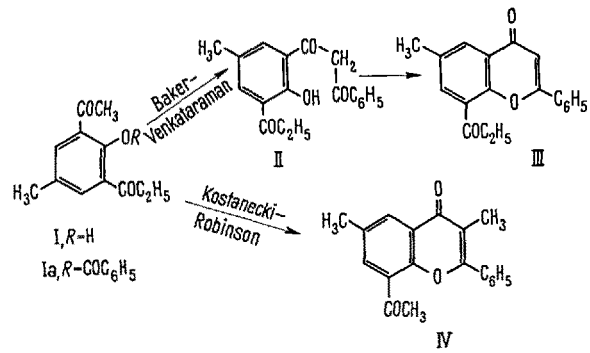
[12] *Acknowledgment*: We sincerely thank Dr. D. M. BOSE, Director, and Dr. P. K. BOSE, Head of the Department of Chemistry of this Institute, for their keen interest and encouragement during the work.

Different Behaviour of 2-Propionyl-4-methyl-6-acetyl-phenol in the Kostanecki-Robinson Acylation and in the Baker-Venkataraman Rearrangement

An identical mechanism seems to be operating in the Kostanecki-Robinson acylation of *o*-hydroxyarylalkylketones and in the Baker-Venkataraman rearrangement of the 0-benzoyloxy derivatives[1,2]. JERZMANOWSKA and MICHALSKA[3] have verified this identity by isolating the corresponding β-diketones from the acylation mixture of 6-methoxy-2-hydroxyacetophenone and benzoic or anisic anhydride. In the course of a research program, diacyl phenols have been investigated and this offered the opportunity to use these intermediates in the above-mentioned procedures. 2-Propionyl-4-methyl-6-acetyl-phenol (I), a compound with two different acyl groups in *ortho* position with respect to the phenolic hydroxyl, was selected in order to detect a hypothetical difference of behaviour. The Kostanecki-Robinson acylation of this product, with benzoyl chloride and sodium benzoate, may be predicted on the basis of a greater tendency of *o*-hydroxypropiophenones as compared to *o*-hydroxyacetophenones to form chromones[4]. In the case of the Baker-Venkataraman rearrangement prediction was doubtful, indeed this transposition occurs equally well either with 0-benzoyloxy-aceto or -propiophenone[5].



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In practice the two reactions produced two different products.

From Ia a flavone m.p. 138–139° (III) (Found: C, 78.10; H, 5.61. Calcd. for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52) from I a different flavone m.p. 222–223.5° (IV) (Found: C, 78.15; H, 5.29. Calcd. for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52) were isolated.

These results must be ascribed to the formation of two different intermediate β-diketones. As the Baker-Venkataraman rearrangement product was yellow, it might be formulated, according to an observation of OLLIS and WEIGHT[5], as ω-benzoyl-2-hydroxy-3-propionyl-acetophenone (II). Consequently it may be inferred that III is 6-methyl-8-propionyl-flavone and IV the corresponding isomer, 3,6-dimethyl-8-acetyl-flavone. These assignments were proved by alkaline hydrolysis of III and IV, which gave 3-propionyl and 3-acetyl-5-methyl-salicylic acids respectively, as well as aceto and propiophenone, characterized as 2,4-dinitrophenyl-hydrazones. The structures of the two salicylic acid derivatives were confirmed by comparing them with authentic samples. The formation of two different flavones therefore, depends on the experimental conditions, i.e. on the different temperatures. At 90°, in the Baker-Venkataraman rearrangement, the migrating benzoyl group is directed to the α-carbon atom of the acetyl chain; at 180–190°, in the Kostanecki-Robinson acylation, the same group is directed to the α-carbon atom of the propionyl chain.

Riassunto. Si riferisce sul diverso comportamento del 2-propionil-4-metil-6-acetilfenolo nella acilazione secondo Kostanecki-Robinson e nella trasposizione di Baker-Venkataraman.

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Breathing Fluid

Foetuses of mammals, including human foetuses, 'breathe' amniotic fluid[1,2]. Newborn mammals may survive complete submersion for considerable periods of time, dependent upon their stage of development. Signs of life have been observed in puppies up to 54 min after submersion in water[3]. Young rats have been reported to continue making respiratory movements for more than 40 min when, shortly after birth, they were submerged in water at 37°C[4]. This tolerance to asphyxia of the newborn, however, diminishes rapidly with age.

It has now been found that adult mammals submerged in a salt solution may breathe fluid for more than 2 h provided they obtain enough oxygen.

Experiments were done on adult white mice. In controls, all respiratory movements ceased approximately 1 min after submersion in saline[5] at 25°C. Animals drowned in 600 ml of saline containing 0.1% of hydrogen peroxide lived from 3 to 5 times as long. Unanesthetized mice submerged in 1500 ml of saline at 25° C which had been saturated with oxygen at 8 atmospheres pressure absolute (8 ata) in a specially constructed transparent tank, continued breathing fluid for periods lasting up to 40 min. Mice anesthetized with pentothal lived up to 2 h and 25 min after submersion in 1500 ml of saline which, after equilibration, initially contained approximately as much oxygen as ambient air at sea level[6].

These experiments clearly demonstrate the potential biological adaptability of adult mammals to a marine environment such as previously existed during ontogenesis and phylogenesis.

Zusammenfassung. Ausgewachsene weisse Laboratoriumsmäuse atmen untergetaucht in 600 ml einer isotonischen Salzlösung bei 25°C 3 bis 5 mal länger, wenn der Flüssigkeit 0,1% Wasserstoffperoxyd zugesetzt wird. In 1500 ml einer isotonischen Salzlösung, die bei einem Sauerstoffdruck von 8 atü (25°C) equilibriert wurde, können sie über 2 h lang Flüssigkeit «atmen».

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Applied Physiology Division, Department of Physiology, University of Leyden (The Netherlands), November 10, 1961.

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Free Amino Acid Pool in Strains of *Shigellae*

The existence of an internal amino acid pool in bacteria has been shown by several investigators[1–10]. MIZUNO et al.[4] first showed the presence of free amino acids within the cells of dysentery bacilli. In the studies on the metabolic activities of members of the genus *Shigella*[11], a number of amino acids were noted in the free amino acid pool of three strains of dysentery bacilli. The composition of the 'pool' of these strains grown in different media is reported in this communication.

The strains used were *Sh. flexneri 2a* (NCTC 8519), *Sh. flexneri 1a* (NCTC 8516), and *Sh. dysenteriae 6* (NCTC 6342) and were chosen because of their different nutritional characters[11]. The minimal medium in which the strain of *Sh. flexneri 2a* showed prompt growth was a chemically defined basal medium[12] supplemented with

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