sor effect had almost disappeared. If the time interval was increased to 40 min, the irregularities became more pronounced. When the time interval was 80 min, complete heart block occurred, with failure of ventricular contraction. KCl administration just before perfusion of epinephrine abolished the arrhythmias.

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Complementary Enzyme Actions in the Clotting of Milk[1]

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According to Berridge (*1*) the clotting of milk by rennin or pepsin is a three-step reaction. The enzyme destabilizes the casein molecule, moderate thermal energy denatures the enzyme-modified molecule, and polyvalent cations crosslink the extended polypeptide chains into a coherent clot. The exact nature of the enzyme action is still obscure.

Below 14° C thermal energy is not sufficient to denature the rennin or pepsin destabilized casein molecule in any convenient time interval. However, if an appropriate protease is present, this denaturation may be brought about enzymatically. For the milk-clotting reaction, this enzymatic denaturing action complements the rennin-like destabilization action.

Many proteolytic enzymes probably can perform both types of enzymatic actions. Examples of this group are pineapple bromelain,[2] papain, and chymotrypsin. Other enzymes at the pH of the milk-clotting test can perform only one of these actions. Thus rennin and pepsin exhibit only the rennin-like destabilizing action at pH 5.3. Trypsin, on the other hand, exhibits principally a protein-splitting action (Table 1). Part of the milk-clotting action of the trypsin sample reported in Tables 1 and 2 may be attributable to chymotrypsin impurities.

If the low temperature milk-clotting ability of pineapple bromelain (Table 2) and papain (*2*) is attributable to two complementary enzyme changes, then it should be possible by combining a rennin-like enzyme with a protein-splitting enzyme to obtain a low-temperature milk-clotting combination. This hypothesis is amply borne out by the data in Table 2. A ratio of 0.44–0.48 milk-clotting units of rennin or pepsin to one protease unit of trypsin gave the best proportions for a low-temperature milk-clotting combination. If a correction is made for the milk-clotting activity of trypsin, the ratio of milk-clotting activity to protease activity is 0.52–0.56.

If similar calculations are made for pineapple bromelain, the ratio of milk-clotting units to protease units is 0.85, quite different from the figure for rennin-trypsin. Furthermore, the addition of either trypsin or rennin to pineapple bromelain increases the unit activity of pineapple bromelain. However, rennin has much more of an activating effect than trypsin.

At the temperature of standard milk-clotting tests, 37.5° C, the thermal denaturation step may proceed more readily than the protein-splitting step (*2*). Hence the standard milk-clotting test would measure principally the rennin-like action of the enzyme. Among different proteolytic enzymes no fixed relation-

TABLE 1

MILK-CLOTTING ACTIVITY AND CASEIN-SOLUBILIZING ACTING OF SEVERAL PROTEOLYTIC ENZYMES AT 37.5° C

Enzyme	Grade	Milk-clotting units*/g	Casein solubilizing units†/g	
			pH 5.3	pH 7.25
Pineapple bromelain	Experimental	4,670	5,500	14,000
Pepsin	N. F.	21,300	Neg.	Neg.
Rennin	N. F. V1	4,270	Neg.	Neg.
Trypsin	N. F.	400	5,000	17,500

* Modified (*2*) Balls and Hoover method (*3*); 5 ml of a 5% milk buffered to pH 5.3.
† Chow-Peticolas method (*4*).

TABLE 2

EFFECT OF COMBINING ENZYMES ON THE TIME NECESSARY TO CLOT 5 ML OF A 5% SKIM MILK SOLUTION AT 4° C

Mg enzyme in 1 ml of added enzyme solution				Minutes for clotting
Bromelain	Trypsin	Rennin	Pepsin	
2.232	—	—	—	12.2
—	4.33	—	—	127.4
—	—	2.24	—	No clot
—	—	—	1.94	No clot
1.116	2.16	—	—	18.0
0.744	2.88	—	—	26.4
0.446	3.46	—	—	35.2
1.116	—	1.12	—	9.3
0.319	—	1.92	—	11.1
0.203	—	2.03	—	19.5
—	3.46	0.45	—	37.1
—	2.88	0.75	—	23.6
—	2.16	1.12	—	17.7
—	1.45	1.49	—	41.2
—	3.99	—	0.15	22.7
—	3.46	—	0.39	16.0
—	3.24	—	0.49	18.0
—	2.88	—	0.64	26.7
—	2.16	—	0.97	30.6

[1] Published with the approval of the Director of the Pineapple Research Institute of Hawaii as Technical Paper No. 214.

[2] For conformity with the rules for naming plant proteolytic enzymes suggested by Greenberg, Winnick, and Lineweaver (*Ann. Rev. Biochem.*, **14**, 31 [1945]) and for the orderly naming of the other enzymes found in the Bromeliaceae, a suggestion has been made to the A.C.S. Committee on Rules, Nomenclature, and Pronunciation that the name pineapple bromelain be substituted for bromelin.

ship would be expected between milk-clotting ability and protein-splitting ability even though the same site on one enzyme may be involved in both of these actions. However, once it has been established that an enzyme preparation consists of only one enzyme having the two actions mentioned and is not a mixture of two enzymes, then the milk-clotting test can be advantageously used with the appropriate correlation factor to assay an enzyme for protein-splitting activity.

The complete explanation of the effect of combinations of enzymes on the low-temperature clotting of milk may provide important clues to the structure of the casein molecule as well as to the mode of action of certain enzymes.

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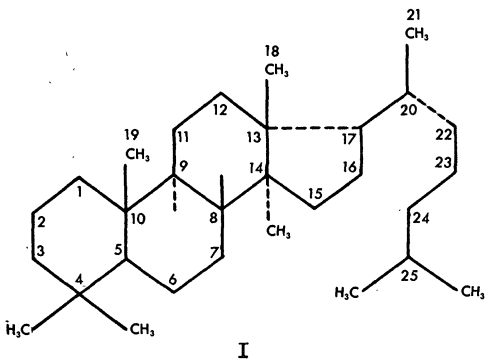
Comments and Communications

Suggested Nomenclatural Revision for "Triterpenoid" Steroids

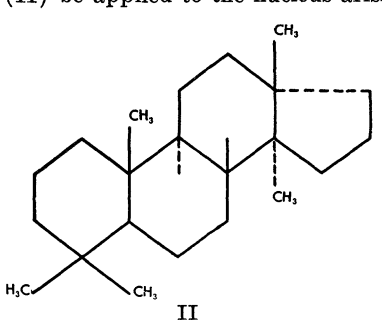
RECENT studies by Ruzicka (*1*) and Barton (*2*) have indicated that the lanostane nucleus may be represented by (I). Systematically (*3*) this may be designated as 4,4′, 14α-trimethylcholestane.



I

It has been noted that the English workers do not use the steroidal numbering system in publications relating to this series of compounds, preferring instead to apply triterpenoid ciphers. This has tended to create confusion where none need exist, as the lanostane series is undoubtedly steroidal in all major respects and should be treated as such.

As an example of the dichotomy which is being practiced, Barton has recently proposed that the name "lanane" (II) be applied to the nucleus arising from lanostane by degradation. There is no particular objection to this new trivial name but the fact that it has been numbered as a terpene seems highly objectionable indeed, since it cannot be construed as anything but 4,4′, 14α-trimethylandrostane and should be numbered as such.



II

Since other nuclei (e.g., euphane) have been shown to be steroidal in nature, it is considered to be particularly important at this time to agree on a consistent nomenclature for the "triterpenoid" steroids. The usages of the Ciba Conference (*3*), though not completely satisfactory, are recommended.

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The Need for a Comprehensive Medical Audio-Visual Aid Center

THE demand for teaching materials of all types is felt in centers of medical activity. Medical schools and institutions receive repeated requests for specimens, models, drawings, photos, exhibits, and various audio-visual aids. One physician asked for a model of coarctation of the aorta to illustrate a lecture before his local medical society. Another did not know where he could obtain anatomic drawings of the components of the mediastinum. A medical student requested a series of x-rays to portray the evolution of lung cancer. An intern wanted to look through a bronchoscope. A resident physician was interested in hearing pericardial friction rubs on a phonographic record. A teacher was setting up an exhibit on electrocardiography and desired electrocardiograms illustrating the common abnormalities. An anatomist sought a three-dimensional model of the renal glomerulus. A history teacher wished to display models of the blood pressure machines in all stages of its development. An inventor