

Citation:

Cohen, N.H., On Lupeol, in:
KNAW, Proceedings, 9 II, 1906-1907, Amsterdam, 1907, pp. 466-470

Chemistry. — “*On Lupeol*”¹⁾. By Dr. N. H. COHEN. (Communicated by Prof. P. v. ROMBURGH).

(Communicated in the meeting of November 24, 1906).

Notwithstanding the many and beautiful researches of several chemists, the structure of cholesterol, which is important also from a physiological point of view, is far from being known. Therefore, Prof. VAN ROMBURGH invited me to investigate a substance closely connected with the same, namely lupeol, a phytosterol. For the phytosterols may be included with the cholesterol in one common group “the cholesterolic substances”. The original intention was to study the alstol found by SACK²⁾ in “bresk”³⁾. From the “bresk” investigated by me, alstol, alstonol and isoalstonol could not be isolated, although SACK claims to have found them in the same, but I obtained α - and β -amyrin and lupeol. It appeared afterwards that SACK’s alstol is not a chemical individual.

Lupeol was first found by LIKIERNIK⁴⁾ in the skins of lupin seeds; afterwards SACK⁵⁾ met with it in the bark of *Rouheria Griffithiana*, whilst VAN ROMBURGH and VAN DER LINDEN⁶⁾ demonstrated its presence as a cinnamate in the resin of *Palaquium calophyllum*. Finally, VAN ROMBURGH proved that TSCHIRCH’s⁷⁾ crystal-albane simply consisted of lupeol cinnamate. The lupeol was prepared from “bresk” by extracting the same first with boiling alcohol. On cooling, a white mass was deposited which, without any further purification was saponified with alcoholic potassium hydroxide. The saponified product was then benzoylated with benzoyl chloride and pyridine and the reaction product treated repeatedly with acetone by heating just to boiling on the waterbath and then filtering off without delay.

Finally, a lupeol benzoate was left, which after repeated recrystallisation from acetone, consisted of fine, flat needles; m.p. 265°—266°, (corr. 273°—274°).

Found C	83.71—83.81	Calculated for $C_{38}H_{54}O_2$	84.07
H	10.41—10.36		10.03

These, like all subsequent combustions, were made with lead chromate.

$[\alpha]_D = +60,75$ in chloroform.

1) For a more elaborate description see Dissertation N. H. COHEN. 1906, Utrecht.

2) SACK. Diss. 1901, Göttingen.

3) Bresk or djetulung is the dried milky juice of some varieties of *Dyera*.

4) Ztschr. f. physiol. Chem. 15. 415 (1891).

5) SACK l. c.

6) Ber. 37. 3440 (1904).

7) Arch. der Pharm. 241. 653 (1903).

By saponification of lupeol benzoate with alcoholic potassium hydroxide and recrystallisation from alcohol or acetone, the lupeol was obtained in the form of fine, long needles m.p. 211°, (corr. 215°).

Found: C 84.62 84.65 84.40 84.50 Calculated for C₃₁ H₅₀ O 84.85
 H 11.78 11.93 11.82 12.02 11.49

$[\alpha]_D = + 27^{\circ},2$ in chloroform.

In the first place it seemed to me of importance to ascertain whether double bonds occur in lupeol. Therefore, a solution of lupeol in carbon disulphide was treated with a solution of bromine in the same solvent. Hydrogen bromide was evolved. By recrystallising the reaction product from methyl alcohol, needles containing 1 mol. of the latter are formed. The melting point of this substance, dried at 100°, was 184°, (corr. 185°).

Found: I	II	III	IV	V	VI	VII	calc. for C ₃₁ H ₄₈ OBr.
C 72.14	72.30						71.90
H 10.26	10.07	CARIUS		LIEBIG			9.55

Br		14.48	14.50	15.40	15.07	14.67	15.45
----	--	-------	-------	-------	-------	-------	-------

$[\alpha]_D = + 3^{\circ},8$ in chloroform.

Most probably, a monosubstitution product had formed and I now tried to obtain an additive product of the benzoate. When dissolved in a mixture of glacial acetic acid and carbon disulphide and then treated with a solution of bromine in glacial acetic acid, it yielded, after spontaneous evaporation of the carbon disulphide, beautiful leaflets. On extracting this product with boiling acetone a less easily soluble substance was left, which proved to be a *monobromide*. After repeated recrystallisation from aethyl acetate, I obtained fine, thick crystals which when melting were decomposed. Placed in the bath at 240° it melted at 243°.

Found I	II	III	IV	V	VI	VII	VIII	IX	X
C 72.62	72.90	72.58	72.46	72.59					
H 8.85	8.88	8.72	9.09	8.84	CARIUS			LIEBIG	
Br					13.14	13.04	12.97	13.40	13.01

Calculated C₃₈ H₅₈ O₂ Br, C = 73.38, H = 8.61, Br = 12.87.

$[\alpha]_D = + 44^{\circ},9$ in chloroform.

The bromine atom is contained in the lupeol nucleus, because on saponification an alcohol containing bromine, and benzoic acid are formed.

The more readily soluble portion crystallises from acetone in beautiful leaflets. It is also a monobromide but could not with certainty be characterised as a chemical individual.

One of the means to trace the structure of a substance is the gradual destruction by oxidation.

The lupeol was, therefore, oxidised with the KILIANI mixture¹⁾. Lupeol dissolved in benzene was shaken with a weighed quantity of the oxidising liquid, 6 atoms of oxygen calculated for 1 mol. of lupeol. Titrations of the oxidising liquid with potassium iodide and sodium thiosulphate showed, that after six hours one atom of oxygen had been consumed and as the amount of chromic acid did not diminish any further, this one atom had been taken up quantitatively. The oxidation product, which crystallised from alcohol in beautiful, thick needles, melted at 169° (corr. 170°) and proved to be a *ketone*, to which I gave the name of *lupeon*.

Found C	84.95	84.91	85.07	84.76	Calc. for C ₃₁ H ₄₈ O	85.24
H	11.64	11.81	11.62	11.61		11.09

$[\alpha]_D = + 63^{\circ},1$ in chloroform.

Dr. JAEGER was kind enough to examine the crystalform of the lupeon. It belongs to the rhombo-bipyramidal class. A complete description will appear elsewhere.

With hydroxylamine an oxime of the lupeon was obtained, which is but little soluble in alcohol.

Recrystallised from ethyl acetate, it forms white, soft, light needles, which are decomposed when melting. Placed in the bath at 278°, they melt at 278°,5.

Found C	81.98	Calc. for C ₃₁ H ₄₈ NOH	82.41
H	11.44	with lead chromate	10.94
N	3.08		3.11

$[\alpha]_D = + 20^{\circ},5$ in chloroform.

Bromine dissolved in glacial acetic acid added to a solution of lupeon in the same solvent gave hydrogen bromide and a dibromide, which was deposited from the acid. Recrystallised from a mixture of benzene and glacial acetic acid it consisted of beautiful, hard needles, which were decomposed when melting. Placed in the bath at 253° the melting point was 254°.

Found	I	II	III	IV	V	VI	VII	VIII	IX	X
C	62.31	62.71	62.50	62.30						
H	8.13	8.26	8.05	8.06	CARIUS			LIEBIG		
Br					26.88	26.91	27.08	26.85	27.35	27.23

Calc. for C₃₁H₄₈O Br₂, C = 62.58, H = 7.80, Br = 26.90.

$[\alpha]_D = + 21^{\circ},4$ in chloroform.

When dissolved in ether, lupeon gave with hydrogen cyanide under the influence of a trace of ammonia a cyanhydrin, which

¹⁾ Ber. 34. 3564 (1901).

after some time deposited in the form of beautiful, thick needles. This substance is decomposed at a higher temperature and also on melting. Placed in the bath at 192° , it melts at 194° . By collecting the hydrogen cyanide liberated on heating in aqueous potassium hydroxide and then titrating with silver nitrate I determined the nitrogen content.

Found:	I	II	III	IV	V	VI	VII	calc. for	$C_{33}H_{62}O_4$	ON
C	82.63	82.76							82.86	
H	11.25	11.26							10.66	
N										3.03

One mol. of cyanohydrin gave, with one mol. of ethyl alcohol and one mol. of hydrogen chloride, a substance, which, when placed in the bath at 230° , melted at 235° ; as shown by a combustion, this was not, however, the expected ethyl ester of the corresponding acid. This substance has not been investigated further.

Lupeol benzoate treated in the same manner as lupeol with the KILIANI mixture was not affected. Lupeon dissolved in benzene and stirred with the mixture for four hours at 40° also remained unaltered.

By the action of chromic anhydride on lupeon at a higher temperature, acid products were formed, which could not be obtained in a crystalline state.

The neutral oxidation product of lupeol with potassium permanganate and sulphuric acid consisted of a mixture, which could be separated only with extreme difficulty. Excepting lupeon no well-defined substance could be isolated from it. As SENKOWSKI¹⁾ had obtained phthalic acid from ebolic acid by oxidation with alkaline permanganate, I treated 23 grams of lupeol in the same manner, but it suffered complete destruction. This fact does, therefore, *not* favour the idea of a *benzene nucleus* in lupeol.

By the oxidation of an acetic acid solution of lupeol acetate with chromic acid, I obtained a product which, on analysis, gave figures which agree satisfactorily with the calculated values for $C_{33}H_{62}O_4$.

Placed in the bath at 285° it melted at 295° to a dark brown mass.

In alcoholic solution this substance did not turn blue litmus red, not even on diluting with water, but still it could be titrated very readily with alcoholic potassium hydroxide, phenolphthalein being used as an indicator. Assuming that one mol. consumes one mol. of KOH the titrations pointed to a molecular weight of 521 and 524, the formula $C_{33}H_{62}O_4$ representing 512.5.

Found:	C	77.59	77.23	76.87	77.24	calculat. for	$C_{33}H_{62}O_4$	77.28
	H	10.75	10.49	10.09	10.79			10.23

¹⁾ Monatsh. f. Chem. 17. 1 (1896).

On saponification with alcoholic potassium hydroxide a substance was obtained which crystallised from ether in needles. Placed in the bath at 260°, the melting point was 263—265°. In regard to litmus this substance behaves like the unsaponified product, but it may be again titrated with alcoholic potassium hydroxide and phenolphthalein. From these titrations the molecular weight was found to be 452 and 461; the formula $C_{31} H_{50} O_3$ represents 470,5.

Found: C 78.42 78.61 calculated for $C_{31} H_{50} O_3$ 79.08
H 11.07 11.05 10.71

The potassium compound of this substance is soluble, with difficulty, in alcohol, and crystallises from this in needles.

On treating either the saponified or the unsaponified oxidation product the same compound was obtained, which seems to be a diacetylated substance. The results of the combustions, however, were not very concordant, but I have not been able to account for this.

Found: C 75.39 74.71 75.67 74.96 74.47 calcul. for $C_{33} H_{54} O_5$ 75.75
H 10.12 10.16 10.51 10.24 9.81

By boiling with excess of alcoholic potassium hydroxide and titrating with alcoholic sulphuric acid the molecular weight was found to be 549, assuming that the molecule contains two acetyl groups. The formula $C_{33} H_{54} O_5$ represents 554.5.

It is desirable to investigate more closely these oxidation products, which are so important in the study of lupeol, before trying to explain their formation.

Lupeol is not reduced by metallic sodium and boiling amyl alcohol; whereas lupeon is reduced by sodium and ethyl alcohol to lupeol. Therefore, if lupeon should possess a double bond, this is sure not to be in $\alpha\beta$ -position in regard to the carbonyl group.

Neither lupeol, nor lupeol acetate dissolved in boiling acetone are acted upon by potassium permanganate. This behaviour does not agree with the theory of a double bond, but the presence of the latter in lupeol and lupeon could be satisfactorily demonstrated by means of HÜBL's iodine reagent. On the other hand the oxidation product $C_{32} H_{52} O_4$ no longer seemed to contain the double bond. On the strength of various combustions and bromine determinations, particularly of dibromolupeon, I consider $C_{31} H_{50} O$ to be the most likely formula for lupeol. The formula $C_{30} H_{48} O$ given by LIKIERNIK ¹⁾ and SACK ²⁾ is certainly not correct.

Utrecht, Org. Chem. Lab. University.

¹⁾ LIKIERNIK l. c.

²⁾ SACK l. c.