Studies on the Constituents of *Anona squamosa L.* (II)[1].

Isolation of (−)-kaur-16-en-19-oic Acid.

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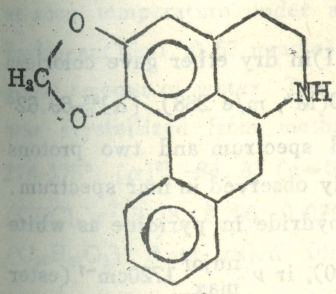
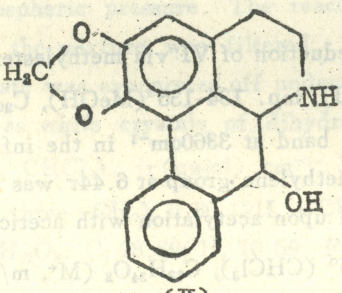
Identification of an unidentified Crystal-H, mp. 175–176° which was isolated from *Anona squamosa* L. (Anonaceae) previously[1] have proved to be a kaurane diterpenoid, (−)-kaur-16-en-19-oic acid (VI) by spectral and chemical evidences. This compound is a plant regulator showing gibberellin-like activity.

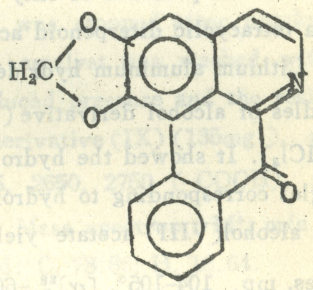
In our systematic investigations of *Anona squamosa L.* (root) led to the isolation of five alkaloids, anonaine(I), michelalbine(II), oxoushinsunine (III), reticuline (IV), and anolobine(V) together with an unidentified non-alkaloidal Crystal-H, mp. 175–176° which were reported in the previous paper[1]. The isolation and characterization of this unkown substance which also obtained from the stem bark of this plant is described in this paper.



(I)



(II)



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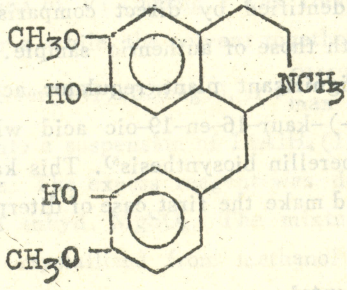
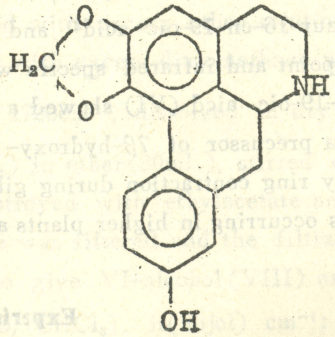


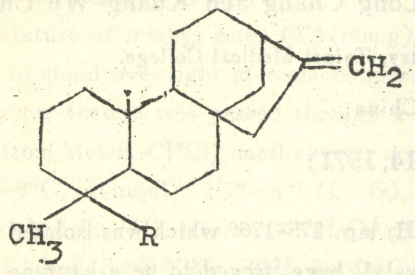
(IV)

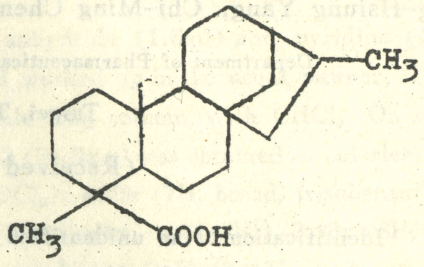


(V)

Crystal-H was crystallized from methanol or acetone as colorless prisms, mp. 175–176°, $[\alpha]_D^{28}$ −109° (c=1, EtOH). It gave no reaction with Mayer's reagent. The elemental analysis

and mass spectrum supported the molecular formula $C_{20}H_{30}O_2$ (M^+, m/e 302), a diterpene derivative. The spectral data showed a characteristic exocyclic terminal methylene group at 3060, 1650, 870cm^{-1} in ir spectrum and 5.30τ in nmr spectrum. The infrared spectra at 1685, 2650. 2750 and 3500cm^{-1} indicated the presence of one carboxylic group, confirmed by nmr spectrum at far downfield -2.05τ. It also appeared two tertiary methyl groups at 9.05 and 8.76τ in nmr signal.





(VI): R=COOH (IX)
(VII): R=$COOCH_3$
(VIII): R=CH_2OH

Catalytic hydrogenation of Crystal-H (VI) with Adam's catalyst (Pt_2O) in glacial acetic acid at room temperature afforded a crystalline compound(IX), mp. 174-175° (MeOH), $[\alpha]_D^{31}$ -84.3° ($CHCl_3$). It appeared a methyl signal at 8.96τ in addition to the original two methyl peaks in nmr spectrum and the end methylene absorption band was absent in ir spectrum. The mass spectrum (M^+, m/e 304) and elemental analysis ($C_{20}H_{32}O_2$) of this hydrogenated product(IX) revealed the presence of only one unsaturated double bond in the molecule, and suggests VI to be a tetracyclic diterpenoid acid.

Lithium aluminum hydride reduction of VI via methylester(VII)in dry ether gave colorless needles of alcohol derivative (VIII), mp. 134-135°(MeOH), $C_{20}H_{32}O$(M^+, m/e 288), $[\alpha]_D^{28}$-69.62° ($CHCl_3$). It showed the hydroxyl band at 3360cm^{-1} in the infrared spectrum and two protons singlet corresponding to hydroxymethylene group at 6.44τ was newly observed in nmr spectrum. The alcohol(VIII) acetate yielded upon acetylation with acetic anhydride in pyridine as white scales, mp. 104-105°, $[\alpha]_D^{28}$ -60.95° ($CHCl_3$), $C_{22}H_{34}O_2$ (M^+, m/e 330), ir ν_{max}^{nujol} 1720cm^{-1} (ester carbonyl). According to the above expermiental data, Crystal-H was assumed as a kauran type diterpenoid, (-)-kaur-16-en-19-oic acid[2] and identified by direct comparison of their tlc, admixture melting point and infrared spectra with those of authentic sample.

(-)-kaur-16-en-19-oic aicd (VI) showed a significant plant regulator activity like gibberellin[3,4] and was a precussor of 7β-hydroxy-(-)-kaur-16-en-19-oic acid which can afford a gibbane aldehyde by ring contraction during gibberellin biosynthesis[5]. This kauren acid is one of the few examples occurring in higher plants and make the first case of diterpene acid isolated from Anonaceae.

Experimental

All melting points are determinated on Yanagimoto micromelting point apparatus and uncorrected. The optical rotations and ir spectra were recorded with Rex Photoelectric Poarlimeter, model NEP-2 and Hitachi Grating IR Spectrophotomer model EPI-G2. The nmr spectra

were obtaind in τ unit with TMS as internal standard in $CDCl_3$. Thin layer chromatography was performed on silical gel F254 (E. Merck) with n-hexane-ethylacetate(7:3) as developing solvent and detection was carried by spraying ceric sulfate in sulfuric acid solution before heating at 105° C for 10 mint.

Isolation:

The isolation procedure of Crystal-H(VI) from n-hexane extract of the root of *A. squamosa L.* was reported previously[1]. This substance was also obtained from stem bark of this plant.

The dried coarsely ground stem bark of *A. squamosa L.* (6.5kg.) were extracted successively with ethyl alcohol for 7 hrs., and the extraction was repeated with fresh charged solvent. The total alcoholic extract was evaporated to dryness under reduced pressure to yield a thick syrup and kept for 2 months. The precipitated solid was collected by filtration and washed with alcohol, then crystallized from methanol as colorless prisms of Crystal-H, mp. 175-176° (12g.). The mother liquid of this compound was investigated in progress.

Crystal-H: (-)-kaur-16-en-19-oic Acid(VI).

Colorless prisms from methanol or acetone, mp. 175-176°, $[\alpha]_D^{28}$ -109° (c=1, EtOH). ir($CHCl_3$) cm^{-1}: 1685, 2650, 2750, 3500 (-COOH), 3060, 1650, 870 ($=CH_2$). nmr($CDCl_3$)τ: 9.05, 8.76(6H, two CH_3), 5.30(2H, $=CH_2$, j=2.5 cps, q), -2.05 (1H, -COOH). Mass spectrum: M^+, m/e 302($C_{20}H_{30}O_2$). Anal. calcd. for $C_{20}H_{30}O_2$. C, 79.42; H, 9.99. Found: C, 79.34; H, 10.25.

Catalytic reduction of VI: dihydro compound(IX).

A suspension of platinum oxide (40mg.) in glacial acetic acid(20ml.) was saturated with hydrogen and VI(202mg., 0.67 mmole) was added. The mixture was hydrogenated catalytically at room temperature under atomospheric pressure. The reaction was stopped after uptake of hydrogen(16ml. 0.67 mmole) and the mixture was filtered. The catalyst was washed with a small amount of water. The filtrate was evaporated off under reduced pressure and the residue was crystallized from methanol as white crystals of dihydro derivative (IX) (135mg.), mp. 174-175°, $[\alpha]_D^{31}$ -84.3° (c=0.875, $CHCl_3$). ir (nujol) cm^{-1}: 1685, 2650, 2750 (-COOH). nmr ($CDCl_3$)τ: 8.78, 8.96, 9.07(9H, three $-CH_3$), 5.30 (2H, $=CH_2$). Mass spectrum: M^+, m/e 304 ($C_{20}H_{32}O_2$). Anal. calcd. for $C_{20}H_{32}O_2$. C, 78.89; H, 10.59. Found: C, 78.86; H, 10.64.

$LiAlH_4$ reduction of VI-methyl ester(VII): VI-alcohol derivative(VIII):

A ether solution of VI (505mg.) was treated with excess ether solution of diazomethane. After standing for a while, the solvent together with excess diazomethane was evaporated off. The oily methyl ester(VII) (520mg.), ir $\nu_{max}^{CHCl_3}$ 1720cm^{-1}, dissolved in dry ether (20ml.) was dropwise added into a suspension of $LiAlH_4$ (1g.) in ether(30ml.), stirred continuously under reflux about 4 hrs. The execss reagent was destroyed with ethylacetate and water, followed by the addition of anhyd. $MgSO_4$. The mixture was filtered and the filtrate was evaporated. The residue was crystallized from methanol to give VI-alcohol (VIII) as colorless needles (465mg.), mp. 134-135°, $[\alpha]_D^{28}$ -69.62° (c=0.675, $CHCl_3$). ir(nujol) cm^{-1}: 3360(-OH), 3050, 1650, 870 ($=CH_2$). nmr ($CDCl_3$)τ: 9.0, 9.05 (6H, two CH_3), 5.28 (2H, $=CH_2$), 6.44 (2H, $-CH_2OH$, q). Mass spectrum: M^+, m/e 288 ($C_{20}H_{32}O$). Anal. calcd. for $C_{20}H_{32}O$. C, 83.27; H, 11.18. Found: C, 83.09; H, 11.57. Acetate: white scales, mp. 104-105° (MeOH), $[\alpha]_D^{28}$ -60.95°

(C=0.52, $CHCl_3$), ir ν_{max}^{nujol} cm^{-1} : 1720(ester C=0), 3050, 1650, 870 (=CH_2). Anal. calcd. for $C_{22}H_{34}O_2$. C, 79.95; H, 10.35. Found: C, 80.03; H, 10.70. Tosylate: colorless crystals, mp. 157–158° (MeOH), $[\alpha]_D^{29}$ -38°, ir ν_{max}^{nujol} cm^{-1} 1590, 1180(tosyl), 3050, 1650, 870 (=CH_2). Anal. calcd. for $C_{27}H_{28}O_3S$. C, 73.30; H, 8.59. Found: C, 73.20; H 8.73.

Acknowledgement

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References

1). T. -H. Yang and C. -M. Chen, J. Chinese Chem. Soc., **17**(4), 243 (1970).
2). C. A. Henrick and P. R. Jefferies, Aust. J. Chem., **17**, 915 (1964).
3). Yutaka Murakami, Shokubutsugaku Zasshi, **81**, 100 (1968); C. A. **68**, 113533y (1968).
4). Stoddart, John L., Phytochem. **8**(5), 831 (1969); C. A., **71**, 19569 (1969).
5). J. R. Hanson and A. F. White, J. Chem. Soc. **8**, 410 (1969); C. A. **71**, 10573u (1969).

中 文 摘 要

蕃荔枝成分之研究（第二報）

(－)-kaur-16-en-19-oic acid 之單離

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關於省產蕃荔枝之成分，著者等曾於前報[1]單離 anonaine (Ⅰ), michelalbine (Ⅱ) oxoushinsunine (Ⅲ), reticuline (Ⅳ) 及 anolobine (Ⅴ) 等五種生物鹼，而今更由其根及幹皮部分離出一種酸性雙萜類之結晶性物質 (Ⅵ), mp, 175—176°, $C_{20}H_{30}O_2$, $[\alpha]_D^{28}$ −109° (EtOH)。經紅外線吸收，核磁共鳴光譜，質譜儀之測定解析及白金接觸還元所得之氫化物 (Ⅸ), mp. 174—175°, $C_{20}H_{32}O_2$; 又與 diazomethane 反應之甲基酯化物 (Ⅶ) 和 $LiAlH_4$ 還元所製備之醇化物 (Ⅷ), mp.134—135°, $C_{20}H_{32}O_2$. Alcohol acetate: mp103—104°; Alcohol tosylate:mp.157—158°.

綜合以上諸光譜分析及化學性質，此酸 (Ⅵ) 直接與標品比較其紅外線吸收光譜，薄層色層分析及混融試驗，確認本品爲 (－)-kaur-16-en-19-oic acid[2] (Ⅵ)，爲一種類似 gibberellins 且具有植物生長激素之作用[3,4]，於生物合成過程中，做爲 gibberellins 之前驅物質[5]。迄今於高等植物中鮮少有被分離證明者，惟由蕃荔枝科植物，證明 (－)-kaur-16-en-19-oic acid 之存在，本研究尚屬首次。