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# PHYTOCHEMICAL STUDIES OF THE BARK OF DIOSPYROS LANCEIFOLIA ROXB(การศึกษาทางพฤกษเคมีของเปลือกต้นฮังฮ้อน)

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ORIGINAL ARTICLE

# PHYTOCHEMICAL STUDIES OF THE BARK OF *DIOSPYROS LANCEIFOLIA* ROXB

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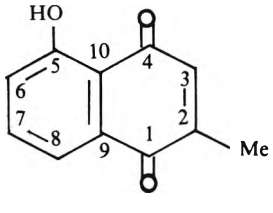
## Abstract

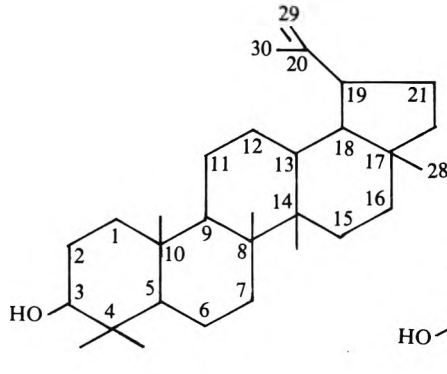
The stem bark of *Diospyros lanceifolia* Roxb. was extracted with hexane successively. A naphthoquinone, plumbagin and two triterpenoids, lupeol and betulin, were isolated and identified. These compounds were compared the melting points, the Rf values on TLC and the spectral data with the authentic samples. (Th. J. Pharm. Sci., Vol.12 No.3, 249-255 (1987)).

## Key Words

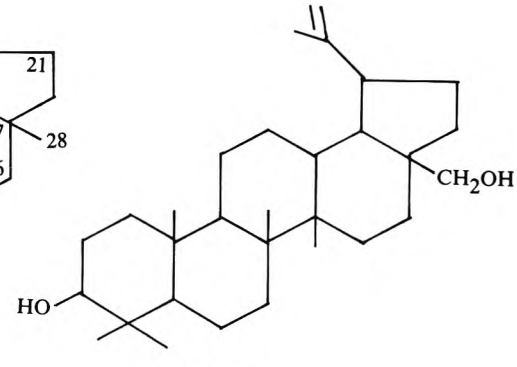
*Diospyros lanceifolia* Roxb., Ebenaceae, naphthoquinone, triterpenoids, lupane, plumbagin, lupeol, betulin.




plumbagin




lupeol




betulin

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## INTRODUCTION

The pan-tropical genus *Diospyros* L. is well known as a source of naphthoquinones, flavonoids and their glycosides, triterpenoids, aromatic acids and lignans. This genus has considerable economic importance as a source of hardwood timbers and of edible fruits (1). These plants have been reported to possess antileprosy (2), antifungal (3), antibacterial (4), antineoplastic (5), termicidal properties (6), which have been attributed to the occurence of naphthoquinones. The extraction of *D. usambarensis* is toxic to *Biomphalaria glabrata* snails. 7-methyljuglone, plumbagin, juglone and isojuglone, the naphthoquinones, represent a very efficient naturally-occuring molluscicide and fungicide (7). Diospyrin, a bis-naphthoquinone, from the bark extract of *D. montana* Roxb., has been reported to inhibit the *in vivo* growth of Ehrlich Ascite Carcinoma, in Swiss albino mice (8).

*D. lanceifolia* Roxb. has been shown to contain both naphthoquinone, plumbagin, and triterpenoids, lupeol and betulin. They were identified on the basis of spectroscopic analysis, notably by $^{1}$H-NMR. The $^{13}$C-NMR spectrum data of plumbagin and betulin are reported.

## MATERIALS AND METHODS

*Source of materials* : The stem bark of *D. lanceifolia* Roxb. was collected from Loey province, Thailand during January 1985. The plant material was identified by Mr. Chamlong Pengklai of the Royal Forest Department, Bangkok.

Melting points were determined by Gallenkamp MFB 595 melting point apparatus and were uncorrected. UV absorption spectra were obtained on doube beam spectrophotometer, Hitachi 220A. IR absorption spectra were determined by Shimadzu 440 using KBr disc. NMR ($^{1}$H and $^{13}$C) spectra were recorded with a Jeol FX-900 in $CDCl_3$ solution with TMS as internal standard. Mass spectra were obtained on a Jeol DX-300/JMA 2000 operating at 70 eV.

## EXTRACTION AND ISOLATION

The dried coarsely powdered bark (600 g) was extracted with hexane (7 L) and concentrated under reduced pressure to give Extract A (2.4 g). The marc was macerated with methanol (7 L), filtered and evaporated *in vacuo* to give syrupy mass which was reextracted with hexane (3 × 200 ml). After concentrating the hexane extract under reduced pressure, it gave 5.35 g of Extract B. Both showed similar pattern on the TLC examination.

The Extract B (1.8 g) was isolated by short column chromatography (silica gel (9385, E.Merck)/ petroleum ether, gradually increase ether from 5 - 50%, ether). The details of the isolation are shown as follows:

| Fraction (25 ml) | Eluent | Compound |
|---|---|---|
| 1 - 7 | 5% ether in petroleum ether | — |
| 8 - 12 | 10% ether in petroleum ether | naphthoquinone, $N_1$ |
| 13 - 24 | 15% ether in petroleum ether | triterpenoid, $T_2$ |
| 25 - 39 | petroleum ether : ether 1:1 | triterpenoids, $T_3$ & $T_4$ |
| 40 - 50 | ether | triterpenoids, $T_3$ & $T_4$ |

The 10% and 15% ether in petroleum ether fractions were concentrated to small volume and kept in a dark place for 2 days furnished orange needle crystal (120 mg) of naphthoquinone designated as, Compound $N_1$ and white-cream needle crystal (100 mg) of triterpenoid designated as, Compound $T_2$, respectively. The petroleum ether : ether 1:1 and ether fractions were combined, concentrated and

rechromatographed over silica gel/chloroform : acetone 5:4. The isolation pattern from column chromatography is shown below.

| Fraction (25 ml) | Eluent | Compound |
|---|---|---|
| | $CHCL_3$ : acetone | |
| 1 - 15 | 5 : 4 | — |
| 16 - 22 | " | triterpenoid, $T_3$ |
| 23 - 30 | " | mixture of triterpenoids |
| 31 - 50 | " | $T_4$ and mixture of triterpenoids |

The eluent from Fractions 16-22 were combined and concentrated to small volume to yield white needle crystal (150 mg) of triterpenoid designated as Compound $T_3$.

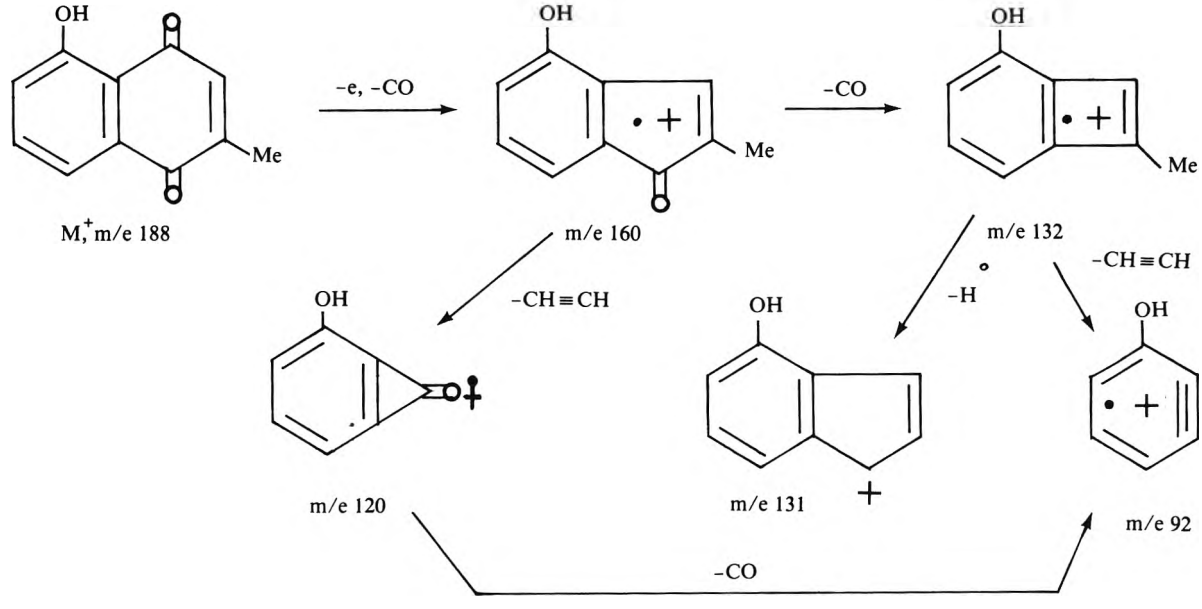
COMPOUND $N_1$ orange needle crystal from petroleum ether, m.p. 74°C (uncorrected), $C_{11}H_8O_3$, TLC : silica gel G (E.Merck) : benzene-chloroform 1 - 3(0.95), chloroform-acetaone 5 - 4(0.97), benzene-petroleum ether 2 - 1(0.34), UV : λ max (EtOH) 268, 426 nm, IR : (KBr) ν max ($cm^{-1}$) 3450 (OH), 2950, 1665 (C=O), 1645 (C=O), 1610 (C=C), 1450, 1365, 1255, 1230, 1160, 1020, 900, 835, 750, $^1$H - NMR : ($CDCl_3$, 90 MHz) δ 2.18 (3H, α, $CH_3$, J = 1.59 Hz), 6.79 (1H, d, $C_3$-H, J = 1.59 Hz), 7.20, 7.58, 7.63 (3H, m, $C_6$-H, $C_7$-H, $C_8$-H), 11.94 (1H, s, $C_5$-OH), $^{13}$C-NMR : ($CDCl_3$, 90 MHz) δ 16.5 (q, $CH_3$), 115.1 (s, $C_9$), 119.2 (d, $C_7$), 124.1 ($C_6$), 132.0 (s, $C_{10}$), 135.4 (d, $C_8$), 136.1 (d, $C_3$), 149.6 (s, $C_2$), 161.2 (s, $C_5$), 184.7 ($C_1$=0), 190.2 ($C_4$=0), MS : 70 eV, m/e (% rel.int.) 189 ($M^+$ + 1, 9.5) 188 ($M^+$, 100), 173 (25), 160 (36.5), 132 (27), 131 (33), 121 (13), 120 (20), 92 (24).

COMPOUND $T_2$ white-cream needle crystal from petroleum ether, m.p. 214°C (uncorrected), $C_{30}H_{50}O$, TLC : silica gel G (E.Merck) : benzene-chloroform 1-3 (0.55), chloroform-acetone 5 - 4 (0.94), petroleum ether-ether 1 - 1 (0.89), UV : λ max (EtOH) 207 nm, IR: (KBr) ν max ($cm^{-1}$) 3350 (OH), 2930, 1640 (C=C), 1450, 1375, 1040, 1010, 880, $^1$H-NMR : ($CDCl_3$, 90 MHz) δ 0.76 (3H, $CH_3$), 0.78 (3H, $C_{28}$-methyl), 0.83 (3H, $CH_3$), 0.94 (3H, $C_{23}$-methyl), 0.96 (3H, $C_{24}$-methyl), 1.03 (3H, $CH_3$), 1.68 (3H, $C_{30}$-methyl), 1.25, 1.38, 1.44, 1.59 (methylenes), 3.16 (1H, m, $C_3$-H), 4.55 (1H, br s, $C_{29}$), 4.67 (1H, br s, $C_{29}$), MS : 70 eV, m/e (% rel.int.) 426 ($M^+$, 51), 411 (16.5), 393 (7), 383 (6), 370 (6), 315 (17), 234 (23), 218 (66), 208 (30), 207 (89), 205 (34), 203 (52), 191 (49), 190 (48), 189 (100), 187 (25), 135 (94), 109 (93)

COMPOUND $T_3$ white needle crystal from ethanol, m.p. 249°C (uncorrected), $C_{30}H_{50}O_2$, TLC : silica gel G (E.Merck) : benzene-chloroform 1 - 3 (0.30), chloroform-acetone 5 - 4 (0.92), petroleum ether-ether 1 - 1 (0.55), UV : λ max (EtOH) 208 nm, IR:(KBr) ν max ($cm^{-1}$) 3450 (OH), 2930, 1643 (C=C), 1483, 1450, 1382, 1365, 1185, 1100, 1080, 1035, 875, $^1$H-NMR ($CDCl_3$, 90 MHz) δ 0.76 (3H, $CH_3$), 0.83 (3H, $CH_3$) 0.98 (6H, $C_{23}$, $C_{24}$-methyl), 1.02 (3H, $CH_3$), 1.69 (3H, $C_{30}$-methyl), 1.25, 1.4, 1.45, 1.6 (methylenes), 3.17 (1H, d, $C_3$-H), 3.31 (1H, d, $C_{28}$, J = 15.3), 3.89 (1H, d, $C_{28}$, J = 15.3), 4.58 (1H, br s, $C_{29}$), 4.68 (1H, br s, $C_{29}$), $^{13}$C-NMR ($CDCl_3$, 90 MHz) δ 14.8 (q, $C_{27}$), 15.4 (q, $C_{24}$), 16.0 (q, $C_{25}$), 16.1 (q, $C_{26}$), 18.3 (t, $C_6$), 19.1 (q, $C_{30}$), 20.9 (t, $C_{11}$), 25.3 (t, $C_{12}$), 27.1 (t, $C_2$), 27.4 (t, $C_{15}$), 28.0 (q, $C_{23}$), 29.2 (t, $C_{21}$), 29.8 (t, $C_{16}$), 34.0 (t, $C_7$), 34.3 (t, $C_{22}$), 37.2 (s, $C_{10}$), 37.3 (d, $C_{13}$), 38.7 (t, $C_1$), 38.9 (s, $C_4$), 40.9 (s, $C_8$), 42.7 (s, $C_{14}$), 47.8 (d, $C_{18}$), 47.8 (d, $C_{19}$), 48.8 (s, $C_{17}$), 50.4 (d, $C_9$), 55.3 (d, $C_5$), 60.6 (t, $C_{28}$-OH), 78.9 (d, $C_3$-OH), 109.7 (t, $C_{29}$), 150.5 (s, $C_{20}$), MS : 70 eV, m/e (% rel. int.), 442 ($M^+$, 30), 427 (8), 424 (11), 411 (52), 399 (10), 385 (11), 234 (33), 220 (24), 207 (70), 203 (68), 201 (24), 191 (41), 190 (33), 189 (100), 187 (31).

## RESULT AND DISCUSSION

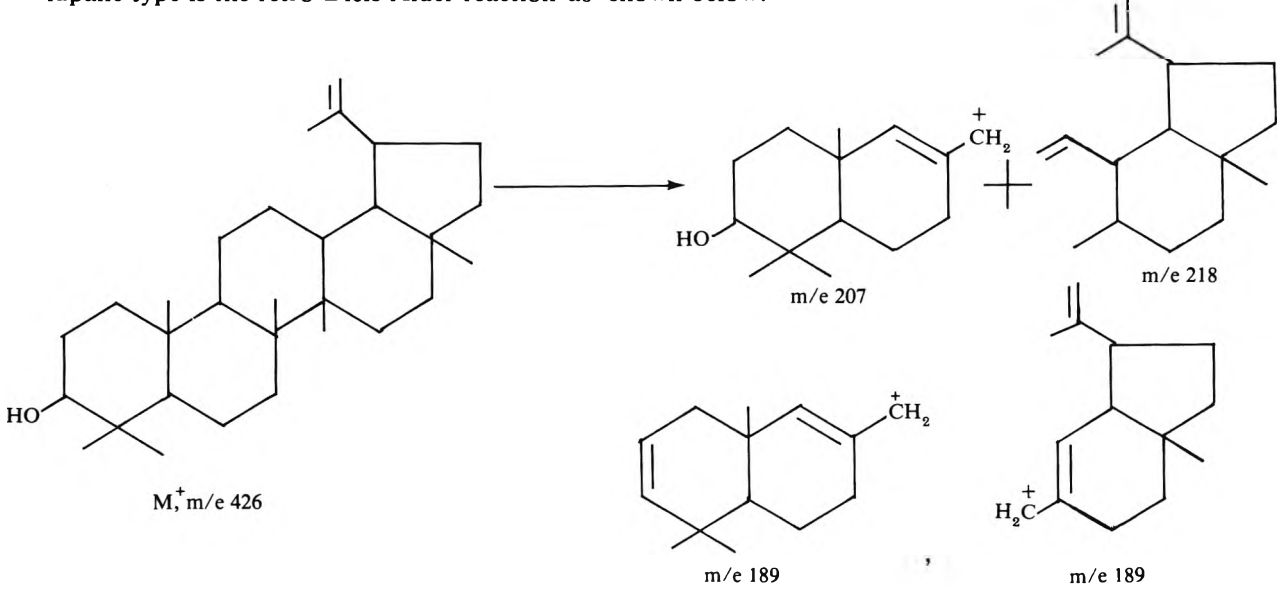
The isolated Compound $N_1$ was a naphthoquinone, it gave red colour with 10% potassium hydroxide solution. The IR spectrum showed hydroxyl group at 3450 $cm^{-1}$, carbonyl groups at 1665 and 1645 $cm^{-1}$, olefin at 1610 $cm^{-1}$ and aromatic ring at 2950 $cm^{-1}$. The $^1$H-NMR showed the presence of methyl group (δ2.18), phonolic (δ11.94) and aromatic protons (δ7.20, 7.58, 7.63). From $^{13}$C-NMR spectrum, there were signals of two carbonyls (δ184.7, 190.2). The mass spectrum, melocular ion peak and base peak of $N_1$ showed at m/e 188 and the main fragmentation is shown below.




M,$^+$m/e 188

Besides the informations from the $^1$H-NMR, $^{13}$C-NMR and Mass spectra, the structure determination of this compound as naphthoquinone, plumbagin was also based on TLC, mmp and IR spectrum comparision with authentic plumbagin which was isolated from some Asian *Diospyros* species (9).

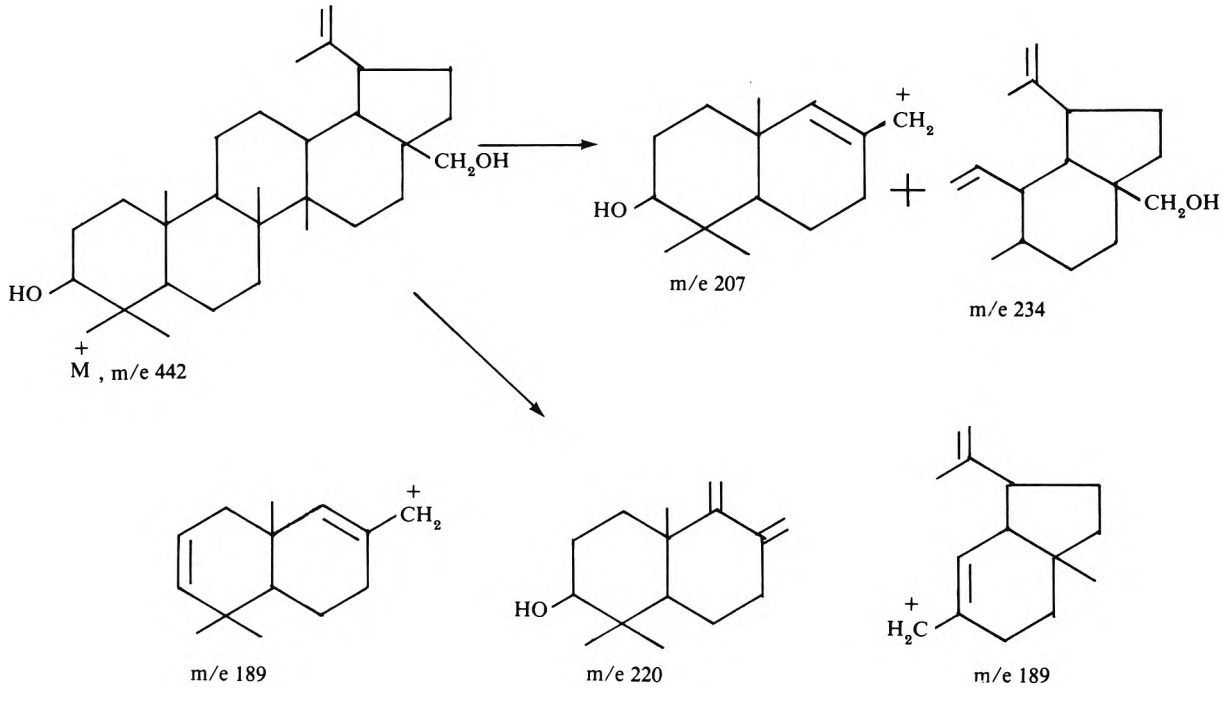
The isolated Compound $T_2$ was a triterpenoid, it gave pink colour with Liebermann Burchard test. The IR spectrum showed the hydroxyl group at 3350 $cm^{-1}$ and olfein at 1640 $cm^{-1}$. The $^1$H-NMR spectrum showed the presence of seven methyl groups (δ0.76, 0.78, 0.83, 0.94, 0.96, 1.03, 1.68) and methylene groups (δ1.25, 1.38, 1.44, 1.59). The mass spectrum, the molecular ion peak and base peak of $T_2$ showed at m/e 426 and 189, respectively. The main fragmentation pathway of this triterpenoid, lupane type is the retro-Diels-Alder reaction as shown below.




M,$^+$m/e 426

The isolated Compound $T_2$ is identified as a known lupane triterpenoid called lupeol by comparison the melting point, Rf values on TLC, and IR spectrum under the same condition with the authentic lupeol which was isolated by Dr. P.G.Waterman (9). Base on spectroscopical data analysis, it is firmly established that $T_2$ is lupeol (10, 11, 12).

The isolated Compound $T_3$ gave positive test with Liebermann Burchard test (violet grey), which classified the compound as triterpenoid. The IR spectrum showed two hydroxyl groups at 3450 $cm^{-1}$, one olefin at 1643 $cm^{-1}$. The $^1$H-NMR spectrum showed signals corresponding to six methyl groups ($\delta$0.76, 0.83, 0.98, 1.02, 1.69) and methylene groups. From $^{13}$C-NMR spectrum, there is a double bond at C-20 and C-29 of isopropylene group ($\delta$150.5 for C-20 and $\delta$109.7 for C-29). Hydroxyl groups attached to C-3 of ring A ($\delta$78.9) and to C-28 ($\delta$60.6). Six methyl groups bonded to carbon atom as was also shown in $^1$H-NMR spectrum ($\delta$14.79, 15.38, 16.03, 16.14, 19.12), 28.0). The mass spectrum, the molecular ion peak and base peak of Compound $T_3$ showed at m/e 442 and 189, respectively. From fragmentation pattern, the presence of $M^+$–43(399) peak, the base peak (189) and the retro-Diels-Alder breakdown at m/e 207, 220, 234 revealed that Compound $T_3$ belongs to triterpenoid, lupane type (10). The main fragmentation pattern is shown below.




The structure identification of Compound $T_3$ as lupane triterpenoid called betulin was mainly based on spectroscopical data analysis (10, 11, 12) and Rf values on TLC, melting point, and IR spectrum comparison with the authentic betulin which was isolated by P.G. Waterman from some Asian *Diospyros* species (9).

The phytochemical studies of minor naphthoquinones and triterpenoids of this species should be done. Further pharmacological studies especially the antineoplastic or anticancer of components isolated from this investigation should be strongly recommended.

## ACKNOWLEDGEMENT

The authors are sincerely greteful to Dr. P.G. Waterman, Phytochemistry Research Laboratory, Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow, United Kingdom for donating the authentic samples.

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# การศึกษาทางพฤกษเคมีของเปลือกต้นฮังฮ้อน



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*รพีพล ภโววาท ปร.ด.***

## บทคัดย่อ

สกัดเปลือกต้นฮังฮ้อนด้วยสารละลายเฮกเซน สามารถแยกและพิสูจน์เอกลักษณ์ของสารได้ 3 สาร ซึ่งเป็นสารกลุ่ม naphthoquinone ที่เรียกว่า plumbagin และสารกลุ่ม triterpenoid อีก 2 สาร คือ lupeol และ betulin สารเหล่านี้ได้เปรียบเทียบค่า Rf บนโครมาโทกราฟีผิวบาง และข้อมูลทางสเปกตรัมกับสารตัวอย่างแท้ (ไทยเภสัชสาร ปีที่ 12(3) : หน้า 249-255 (2530)).

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