Petri plate). The poured material was allowed to gel. After gelling the medium, pores were made using a sterile cork borer and scooping out the punched part of the agar. Into these wells were added 0.05 ml portions of the test compound in solvent. The drug solution was allowed to diffuse for about an hour into the medium. The plates were incubated at 37° for 48 h. DMSO was taken as control to know the activity of solvent. The standard drug ciprofloxacin was also screened under similar conditions for comparison. The results for the antibacterial screening are presented in Table 1.

It has been observed that some of these compounds exhibited interesting antibacterial activities. Results reveal that compound 4b and 4d were active against both gram positive and gram-negative bacteria where as compound 4c and 4f were active against *E. coli* and *B. cerus*. Antibacterial data indicated that compound 4a and 4e did not show any significant antibacterial activities. It has been observed that activity shown by compound 4g against *P. aeruginosa* was comparable to that of standard drug. The screening results indicated that compounds 4a-4f (except 4g) were mild to moderately active against *E. coli* at a concentration of 1 mg/ml.


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# Synthesis and Antiinflammatory Activity of Oleanolic Acid Hemiphthalate Disodium Salt


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**A simple method for synthesis of oleanolic acid hemiphthalate disodium salt has been successfully developed. The structures of the newly synthesized compounds were elucidated on the basis of analytical and spectral data. The antiinflammatory activity of oleanolic acid hemiphthalate disodium**



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**salt was performed on dimethyl benzene-induced acute ear oedema model. In the acute inflammatory model, oleanolic acid hemiphthalate disodium salt exhibited significant anti-inflammatory activity with single and multiple doses with no matter oral, or subcutaneous or intraperitoneal administration. Furthermore antiinflammatory activity of oleanolic acid hemiphthalate disodium salt was better than oleanolic acid with single dose with various administration methods and with multiple doses with oral administration method.**

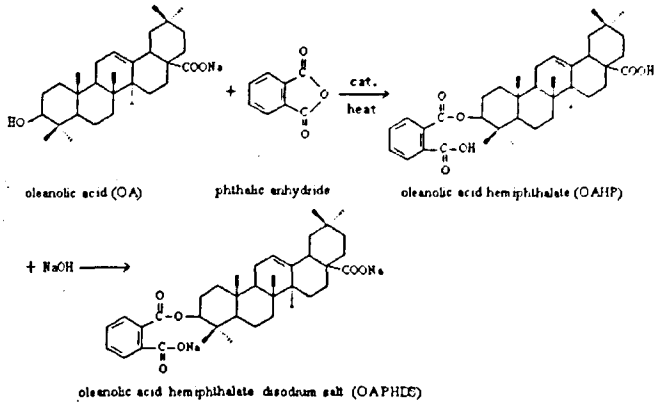
In general, there are many chemical constituents including effective constituents and auxiliary constituents in Traditional Chinese Medicine (TCM). Auxiliary constituents are supposed to assist the effective constituents in dissolution and absorption. For example, saponin in TCM can be considered as an auxiliary constituent since it is found to act as surfactant and aids in dissolution of other effective constituent. It will cost much time and much money to study auxiliary constituent, especially to study their mechanism of action. So the author put forward a new idea as follows: on the basis of the studies on the effective constituent by predecessor, we modify the effective constituent obtained from TCM into drug-like prodrug having better pharmacological effects than it. It might be due to the fact that prodrug plays two roles of auxiliary constituents and effective constituents.

Oleanolic acid (OA) exists widely in herbs that are listed especially in Chinese materia medica. It has hepatoprotective[1], antiinflammatory[2] and antitumor effects[3]. OA cannot be absorbed completely due to its poor solubility, which induces low bioavailibility. OA is chosen as example in this study according to the above idea because OA has not only good pharmacological activity but also no side-effects. OA was modified into oleanolic acid hemiphthalate disodium salt (OAHD) that has better solubility than OA. Then acute antiinflammatory activity of OAHD was evaluated by dimethyl benzene-induced ear edema method.

OA was bought from Huanghe Pharmaceutical factory, China; hydrocortisone (HYD) was provided by the National Institute for the Control of Pharmaceutical and Biological Products of China. Melting points were determined on an electrothermal apparatus in open capillary tubes. The IR spectra were recorded on a Shimadzu (Japan) model IR-300 spectrometer. The electronic absorption spectra were recorded on Beckman Du-65 UV spectrophotometer. The $^1H$ NMR spectra were recorded on a Bruker Am-400 NMR spectrometer, using tetramethyl silane as internal standard (chemical shifts expressed in ppm). The mass spectra were recorded on a Bruker DX-300 mass spectrometer.

Swiss mice of either sex (18-22 g) were obtained from the animal center of Shenyang Pharmaceutical University. All animals use procedures were in accordance with the regulations of experimental animal administration issued by the state Committee of Science and Technology of the People's Republic of China in November 14th 1988. They were housed in cages at 25±2°, relative humidity of 45~55%, maintained under 12 h light and dark cycle and were fed with standard animal feed. All animals were acclimatized for a week before use.

Synthesis of the title compounds is depicted in scheme 1. OA (5 g) and phthalic anhydride (2.0 g) was dissolved in dry pyridine (150 ml). The reaction mixture was refluxed for about 12 h in oil bath with good stirring by magnetic stirrer. The excess of solvent was removed by distillation. The resultant solid was washed with hot distilled water, filtered, dried and recrystallized from ethanol-dichloromethane (1:1) to obtain the white crystals oleanolic acid hemiphthalate (OAHP). Yield: 94.6%, m.p. 254~258¡ã(disintegrated), Anal. calcd. for $C_{38}H_{52}O_6$ C 75.46%, H 8.67%. found, C 75.57, H 8.78, TLC: cyclehexane-acetone-ethylacetate-formic acid (5:2:0.5:0.1), Rf (0.41); UV(MeOH) $\lambda_{max}$ nm: 237, 278. IR (KBr) ν $cm^{-1}$: 1710 (Ar-COOR $\nu_{C=O}$), 1270 ($\nu_{as\ C\text{-}O\text{-}C}$), 1545 (COO-,$\nu_{as\ C=O}$), 1560 ($\nu_{as\ C=O}$), 1395 ($\nu_{s\ C=O}$); FAB-MS m/s: 605($M^+$+1), 558, 439, 393; $^1H$ NMR ($CDCl_3$) δ ppm: 4.68~4.72




**Scheme 1: Synthesis of OAHD.**

(m£¬$C_3$-H), 5.26 (s, $C_{12}$-H), 7.52~7.85 (m, Ar-H); $^{13}C$ NMR ($CDCl_3$) δ ppm: 83.13 (C-3), 122.60 (C-12), 143.57 (C-13), 184.78 (C-28), 167.81 (COOR), 172.61 (COOH).

OAHP was dissolved in 50 ml of ethanol, into which 30% of sodium hydroxide solution was added until the pH value reach to 10, and then OAHPDS was recrystallized slowly from the ethanol solution. Yield: 96.5%, m.p. 290¡ã(disintegrated), UV (MeOH) $\lambda_{max}$nm: 240, 273, IR (KBr) ν $cm^{-1}$: 1720 (Ar-COOR $\nu_{C=O}$), 1290 ($\nu_{as\ C\text{-}O\text{-}C}$), 1715 (Ar-COOH, $\nu_{C=O}$),1695(COOH, $\nu_{C=O}$),1605,1580,745 (CH, aromatic ), 1650 ($\nu_{C=C}$); The saturated aqueous solubility of OAHD was determined as 33.3 mg/ml (25°), much bigger than that of OA (4.61 μg/ml, 25°)[7].

Acute antiinflammatory activity of OAHPDS was evaluated by dimethyl benzene-induced ear edema method. Swiss mice of either sex (18~22 g) selected by random sampling technique were employed for the study. Each group consisted of ten animals. Their right ears were smeared with 20 μl of dimethyl benzene on both sides while left ears were not smeared as control. OAHD or OA was administered with single dose as a solution and suspension in 1% Tween-80 intra-gastrically, subcutaneously and intraperitoneally at three dose levels (25, 50, 100 mg/kg), respectively, 1 h prior to dimethyl benzene administration. The mice were sacrificed by dislocating cervical vertebra at 1 h after dimethyl benzene administration. Then the left and right ears were cut out same area at same region with hole-maker (diameter is 9 mm) and were weighed. 0.1 milliliter of 1% tween-80 served as control and hydrocortisone (HYD) (20 mg/kg) as standard. The degree of oedema was calculated as following equation: The degree of oedema (mg) = right ear weight cut out (mg)– left ear weight cut out (mg). The other experiments were carried out after multiple doses administrations (once a day for 7 d).

A simple method for synthesis of OAHD has been successfully developed. OAHD synthesized was confirmed by mp, elemental analyses and spectral data. The presence of two C=O of phthalic acid stretching frequency was found as sharp absorption band near 1720 $cm^{-1}$ and 1715 $cm^{-1}$. The peaks due to phenolic proton was observed as δ7.52~7.85 in $^1H$ NMR spectrum of OAHD. The strong evidence of the synthesis reaction obtained as $M^+$ peak at 605 m/z of mass spectrum corresponding to the molecular weight of the structure postulated.

The results showed in Table 1 and Table 2 that OAHD exhibited significant antiinflammatory activity at various administration methods no matter with single dose or with multiple doses. Furthermore anti-inflammatory activity of OAHD was better than that of OA with single dose with various administration methods and with multiple doses with oral administration method. It is inferred that the modification of structure did not affect the antiinflammatory function of OA, but its physicochemical property, like aqueous solubility resulting in quick onset.

TABLE 1: ACUTE ANTIINFLAMMATORY ACTIVITY OF OAHD COMPARED WITH OA WITH SINGLE DOSE

| Group | Dose mg/kg | The degree of oedema (mg) | | |
|---|---|---|---|---|
| | | ip | sc | ig |
| Control | | 12.4±2.84 | 12.5±2.35 | 12.7±2.06 |
| OA | 25 | 11.4±1.67 | 11.2±1.48 | 10.6±3.84 |
| OA | 50 | 10.2±2.76 | 11.0±2.48 | 11.1±1.56 |
| OA | 100 | 10.5±3.06 | 10.5±1.85 | 10.8±2.42 |
| OAHPDS | 25 | 9.26±2.77 | 9.23±2.72 | 9.61±2.78 |
| OAHPDS | 50 | 8.41±2.10* | 8.36±2.13* | 8.60±1.95* |
| OAHPDS | 100 | 8.06±1.96* | 8.17±2.09* | 7.06±2.30* |
| HYD | 20 | 4.40±1.71* | 4.37±1.69* | 4.84±2.05* |

Ip, sc and ig represent intraperitoneally, subcutaneously and intra-gastrically administration, respectively. Each value represents mean±standard error of the mean of 10, Each group consisted of 10 animals. Asterisk indicates level of significance compared with control, *$p<0.05$ (student' test). HYD was intramuscularly administration in every group

TABLE 2: ACUTE ANTIINFLAMMATORY ACTIVITY OF OAHD COMPARED WITH OA WITH MULTIPLE DOSES

| Group | Dose mg/kg | The degree of oedema (mg) | | |
|---|---|---|---|---|
| | | ip | sc | ig |
| Control | | 12.6±2.72 | 13.0±2.47 | 12.5±1.99 |
| OA | 25 | 8.93±1.87* | 8.94±2.09* | 10.3±1.49 |
| OA | 50 | 6.80±1.10* | 7.60±1.67* | 9.23±2.41* |
| OA | 100 | 6.00±1.57* | 6.25±1.95* | 8.00±3.37* |
| OAHPDS | 25 | 10.0±3.74 | 10.5±3.58 | 7.45±2.18* |
| OAHPDS | 50 | 8.05±2.64* | 8.12±2.37* | 6.60±2.83* |
| OAHPDS | 100 | 7.50±1.81* | 7.42±1.59* | 5.64±2.10* |
| HYD | 20 | 4.52±2.08* | 4.44±1.97* | 4.41±2.09* |

Ip, sc and ig represent intraperitoneally, subcutaneously and intra-gastrically administration, respectively. Each value represents mean±standard error of the mean of 10, Each group consisted of 10 animals. Asterisk indicate level of significance compared with control, *p<0.05 (student' test). HYD was intramuscularly administration in every group.

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# Antitumour Property of Vinblastine Monohydrazide


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**The bioconverted product of vinblastine (alkaloid from *Vinca rosea*), vinblastine monohydrazide was administered to cell line-induced solid tumour in mice and the changes in life span and tumour size were noted. It was found that the bioconverted product was an antitumour agent as it**



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